General and Comparative Endocrinology 279 (2019) 12–26

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Reproductive hormones modulate differentially brain and ovarian vasotocin receptor gene expression in early and late recrudescent catfish, *Heteropneustes fossilis*

A. Rawat[a], R. Chaube[a,**], KP. Joy[b,*]

[a] Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi 221005, India
[b] Department of Biotechnology, Cochin University of Science and Technology, Kochi 682022, India

ARTICLE INFO

Keywords:
Catfish
Vasotocin receptor gene expression
Steroid hormones
Gonadotropin
Reproductive phases

ABSTRACT

Investigations on the role of the reproductive hormones on VT receptor gene expression are lacking in teleosts. Previously we reported that gonadotropin and steroid hormones modulate the secretion and gene expression of brain and ovarian vasotocin (VT) in the catfish *Heteropneustes fossilis*. In continuation, in the present study we investigated the role of estradiol-17β (E_2), the maturation-inducing steroid (MIS) 17α, 20β-dihydroxy-4-pregnen-3-one (17, 20β-DP), and human chorionic gonadotropin (hCG) on the expression of VT receptor genes (*v1a1*, *v1a2* and *v2a*) in the brain and ovary of the catfish in early (previtellogenic, preparatory) and late (post vitellogenic, prespawning) phases of the ovarian cycle. The steroid treatments (*in vivo* and *in vitro*) modulated only the *v1a1* and *v1a2* expression in both tissues, but not the *v2a* expression. The E_2-induced modulation of the *v1a1* and v1a2 gene expression varied with the reproductive phase. In the preparatory phase, E_2 up regulated the expression of brain and ovarian *v1a1* and *v1a2* gene expression, the response varied with the dose and duration. In the prespawning phase, E_2 inhibited the expression in a dose- and duration-dependent manner. On the other hand, 17, 20β-DP up regulated the expression of brain and ovarian *v1a1* and *v1a2* in both phases, and the response was higher in the prespawning phase and varied with dose and duration. In contrast to the steroid effects, the hCG treatment modulated the expression of all the VT receptor genes only in the prespawning phase and the response varied with dose and duration. The results indicate differential modulatory roles of steroid hormones and hCG on the VT receptor gene expression, to mediate VT's reproductive or osmoregulatory functions. While the hCG effect on v1a type receptor expression may be steroid- dependent, that of *v2a* expression seems to be steroid-independent.

1. Introduction

The vasopressin homolog vasotocin (VT) is thought to be the evolutionary forerunner of all vertebrate neurohypophysial (NH) nonapeptides (Acher, 1996) and is equally potent in the control of osmoregulation and reproduction. Many of the better characterized peripheral physiological functions of VT are brought about by its ability to contract smooth muscles (Amer and Brown, 1995; Wells et al., 2002). In the brain, centrally released VT assumes the role of a neuromodulator or neurotransmitter that influences social and reproductive behaviors (Salek et al., 2002; Goodson and Bass, 2000; Semsar and Godwin, 2003). In addition to the well established functions of osmoregulation and reproduction, VT is involved in other functions such as metabolism, maintenance of circadian and seasonal calendar to stress response (Balment and Warne, 2006). These diverse functions are mediated by at least three receptor subtypes which show a wide tissue distribution (Lema, 2010; Lema et al., 2012). Two of them belong to the mammalian V1a subfamily and are named v1a1 and v1a2 subtypes, and the third is the v2a type (Ocampo Daza et al., 2012; Yamaguchi et al., 2012; Lagman et al., 2013). The mammalian V1a and V2 classes of receptors employ, respectively, the protein kinase C and protein kinase A signaling pathways to mediate various physiological processes (Gimpl and Fahrenholz, 2001). In the catfish *Heteropneustes fossilis*, three VT receptor subtype genes, viz., *v1a1*, *v1a2* and *v2a* were cloned and characterized, and the gene transcripts showed wide tissue distribution (Rawat et al., 2015). The *v1a1* and *v1a2* transcripts have extensive and overlapping distribution in different brain regions and the pituitary while *v2a* transcripts have limited localization (Rawat et al.

* Corresponding author.
** Co-Corresponding author.
E-mail addresses: chauberadha@rediffmail.com (R. Chaube), kpjoybhu@gmail.com (K. Joy).

https://doi.org/10.1016/j.ygcen.2018.06.018
Received 2 April 2018; Received in revised form 18 June 2018; Accepted 27 June 2018
Available online 28 June 2018
0016-6480/ © 2018 Elsevier Inc. All rights reserved.

unpublished data). Similarly, the VT receptor gene paralogs have a differential distribution in the ovary. Both *v1a1* and *v1a2* are localized in the follicular layer composed of the endocrine theca – granulosa while the *v2a* mRNA is localized in the cytoplasm and zona radiata (granulosa – oocyte membrane complex).

In the previous study, Singh and Joy (2008) reported the distribution of VT in the hypothalamo-hypophysial-ovarian axis of the catfish *H. fossilis* using VT-specific antibodies. VT- immunoreactivity was obtained in the neurons of the nucleus preopticus (NPO), in the neurohypophysis of the pituitary and follicular layer of the ovarian follicles. VT showed significant seasonal changes in the brain, plasma and ovary with high levels in the brain in preparatory phase but high titers in the plasma and ovary in spawning phase. In subsequent studies, a direct role of VT in ovarian steroidogenesis, oocyte maturation and hydration, ovulation and prostaglandin synthesis has been reported in the catfish *H. fossilis* (Singh and Joy, 2009a, 2010, 2011; Joy and Singh, 2013; Joy and Chaube, 2015). VT differentially modulated estradiol-17β (E_2) in a biphasic manner (low doses stimulated and high doses inhibited) in the preparatory (previtellogenic) phase while VT inhibited E_2 in the post-vitellogenic ovary (prespawning and spawning phases). On the other hand, VT stimulated consistently progestin steroids: P_4, $17P_4$, and 17α, 20β- dihydroxy4-pregnen-3-one (17, 20βDP- maturation-inducing steroid) in all the three phases; the effect was maximal in the spawning phase. Similarly VT stimulated germinal vesicle breakdown (meiotic maturation), oocyte hydration, *aquaporin-1ab* expression (Acharjee et al, 2011), prostaglandin ($PGF_{2\alpha}$ and PGE_2) and ovulation. In all these, the effects of VT were greater than the neutral peptide, Isotocin and similar to human chorionic gonadotropin (hCG). VT is considered a co-gonadotropin in the catfish. Estrogens and progestins modulate VT levels: estradiol-17β (E_2) elicits a reproductive-stage- specific stimulatory or inhibitory effect while progestins exert a stimulatory effect (Singh and Joy, 2009b). Human CG with LH activity and Ovaprim (salmon GnRH analogue and domperidone, a dopamine-2 receptor blocker) have been used to induce ovulation in fishes and VT showed periovulatory changes with the peak increase at 16 h post injection when eggs are stripped and fertilized (Singh and Joy, 2009b; Singh and Joy, 2011). Thus, the catfish ovary has a full complement of the VT system, as in the brain-pituitary.

Since the aforesaid actions of VT are inhibited by either mammalian V1 type or V2 type receptor blockers (references as above), we were interested to know whether the reproductive hormones modulate VT receptor expression. The effects of E_2, 17, 20β-DP and hCG on the expression of the VT receptor genes were investigated *in vivo* in the brain and both *in vivo* and *in vitro* in the ovary in the previtellogenic (preparatory phase) and post vitellogenic (prespawning phase) female fish.

2. Material and methods

2.1. Animal collection and acclimatization

Heteropneustes fossilis were collected from local fish markets in the preparatory (March) and prespawning (June) phases of the reproductive cycle. Female fish were sorted out and a few fish were sampled to determine the ovarian stage. The gonado-somatic index (GSI) was 0.48 ± 0.03% in the preparatory phase (previtellogenic stage) and 9.05 ± 0.1% in the prespawning phase (post vitelloegenic stage). The fish were maintained in the laboratory for a week under natural photoperiod and temperature (11.5L: 13.5D; 22 ± 2 °C and 13L: 11D; 26 ± 2 °C, respectively) to overcome stress due to transportation and were fed with boiled goat liver *ad libitum*.

The experiments were performed in accordance with the guidelines of Animal Ethics Committee of Banaras Hindu University for experimentation in animals and care was taken to prevent cruelty of any kind.

2.2. Chemicals

Estradiol-17β (E_2) and 17α, 20β-dihydroxy-4-pregnen-3-one (17, 20β-DP) were purchased from Sigma chemical company, St. Louis, USA. Human chorionic gonadotropin (Corion; IBSA; Switzerland) was purchased from a local medical store. RNeasy lipid tissue mini kit (Qiagen), Revert-Aid H Minus first strand cDNA synthesis kit (Fermentas), DNase I RNase-free (Ambion) and veriquest SYBR green qPCR master mix (Affymetrix) were procured through local suppliers. Agarose, tris base, glacial acetic acid, EDTA–Na2 and other chemicals were of molecular grade, and purchased locally. Primers were synthesized by Integrated DNA Technologies, Faridabad, India.

2.3. In vivo experiments

In each phase, 24 groups of 5 acclimated fish each were used for the steroid experiments. E_2 and 17, 20β-DP were weighed and dissolved in 50 μL ethanol in a dark bottle and diluted with fish saline (0.6% NaCl) and kept at 4 °C. E_2 (0.05, 0.1 and 0.5 μg/g BW) and 17, 20β- DP (0.1, 0.5 and 1.0 μg/g BW) were administered intraperitoneally at the base of pectoral fin. Six groups of 5 fish each were given the vehicle (0.6% NaCl containing 50 μL ethanol), as controls. The fish were sampled at 8, 16 and 24 h. Brain and ovary were dissected out and stored in RNA later at −80 °C until processed for the gene expression assay. For the hCG experiment, 13 groups of 5 acclimatized fish each were used in each phase. Human CG was dissolved in fish saline and was injected intraperitoneally at doses of 20, 50 or 100 IU each. The fish were sampled at 8, 16 and 24 h. As controls, six groups of 5 fish each were given fish saline. Brain and ovary were dissected out and stored in RNA later at −80 °C until processed for the gene expression study.

In the hCG experiment, five fish in each group were checked for ovulation by manual stripping at 0, 8, 6 and 24 h and scored for determining ovulation.

2.4. In vitro experiments

2.4.1. Preparation of incubation medium and test compounds

Incubation medium was prepared as follows: NaCl-3.74, KCl-0.32, $CaCl_2$-0.16, $NaH_2PO_4.H_2O$-0.10, $MgSO_4$-$7H_2O$-0.16 and glucose −0.40 (weights in g) were dissolved in 1 L of triple distilled water. The incubation medium was autoclaved and pH was adjusted to 7.5 with 1 M sodium bicarbonate. Penicillin (2,00,000 IU) and streptomycin sulphate (200 mg) were added and filtered. The medium was stored at 4 °C and prepared fresh every week. The hormone stock solutions were prepared as described above. Just before the incubation, the stock solutions were diluted with the incubation medium to make working concentrations.

2.4.2. Incubation of ovarian tissue

In the preparatory and prespawning phases, ovaries were collected from adult acclimatized female fish, weighed and transferred into a sterile Petri dish containing freshly prepared cool incubation medium. Ovary pieces (about 100 mg each) were rinsed and transferred to culture plate wells containing 5 mL medium each with different concentrations of E_2 (1, 10 and 100 nM), 17, 20β-DP (1, 10 and 100 nM), and hCG (20, 50 and 100 IU) and incubated at 22 ± 2 °C for 8, 16 or 24 h. For controls, the ovarian pieces were incubated with the medium containing the same amounts of vehicle or fish saline. All incubations were done in triplicate. Five replicates were used for each concentration of the test compounds. The medium was changed after every 4 h to maintain a constant pH. After completion of the incubation, the tissues were stored in RNA later at -80 °C, until processed for gene expression studies.

2.4.3. Determination of GVBD in vitro

In the prespawning phase, sexually mature female *H. fossilis* were sacrificed by decapitation and ovaries were transferred to a Petri dish

containing fresh cooled incubation medium. Round, dark green post-vitellogenic ovarian follicles (1 mm diameter) with centrally-located germinal vesicle (GV) were selected and separated with fine brush and watchmaker's forceps. Batches of about 50–60 follicles were incubated in triplicate in embryo cups containing 3 mL incubation medium containing the vehicle or medium containing test hormones: 17, 20β-DP (1, 10 and 100 nM) or hCG (20, 50 and 100 IU) at 22 ± 2 °C for 0, 8, 16 and 24 h. The incubations replicated with follicles from 5 fish ovaries. The medium was changed every 4 h and replenished with fresh medium containing required amount of the test compound.

At the end of the incubations, the follicles were cleared in a clearing solution (ethanol: acetic acid: formalin, 6:1:3) and observed under a stereobinocular microscope. Translucent follicles without GV were scored. Percentage of GV breakdown (GVBD) was calculated as,

$$\frac{\text{Follicles that underwent GVBD}}{\text{Total number of follicles incubated}} \times 100$$

2.5. qPCR assay

Total RNA was extracted from the tissues (100 mg) stored in RNA later by the single step method of RNA isolation. RNA purity was checked by calculating A260/A280 ratio. Samples having a ratio above 2.0 were only used. Absence of genomic DNA contamination in the RNA was confirmed by using non-reverse transcribed samples as templates. In addition, the absence of DNA in total RNA was ensured by treating with DNAse I before proceeding for the first strand cDNA synthesis. Five μg of total RNA was reverse transcribed using random hexamer primers and Revert Aid M-MuLV reverse transcriptase in a 20 μL reaction volume (first strand cDNA synthesis kit, Fermantas), using the manufacturer's protocol. Gene-specific primers were designed for *v1a1, v1a2* and *v2a* from the respective sequences (Table 1). Primers for β-actin were used as the internal control. The specificity of each primer pair was confirmed by dissociation curve analysis. The qPCR assays were performed in triplicate for different samples using the specific primers and VeriQuest ™SYBR Green qPCR master mix with ROX (Affymetrix, Inc. Cleveland, Ohio USA) in a ABI Prism 7500 thermal cycler (Applied Biosystems, Foster, CA, USA) at 95 °C (15 s) and 60 °C (1 min) for 40 cycles. Each sample was run in a final volume of 20 μL containing 1 μL of cDNA, 10 pM of each primer, and 10 μL of SYBR Green PCR master mix. Specificity of amplicons was verified by melting curve analysis (60–95 °C) after 40 cycles. As controls, the assays were performed without templates. No amplification was observed in the control studies. Cycle threshold (Ct) values were obtained from the exponential phase of PCR amplification and target gene (*v1a1*, *v1a2* and *v2a*) expression was normalized against the β-actin gene expression to generate a Ct value. Comparative Ct (ΔΔCT) method (Livak and Schmittgen, 2001) was used to quantify the target gene abundance. The β-actin standard gave consistent amplification in our assay system.

2.6. Statistical analysis

Data were expressed as mean ± SEM (n = 5) and checked for homogeneity and normality distribution. In Levene's test, the error variances of the dependent variables were equal across groups ($p > 0.05$) and the data followed a normal distribution in Kolmogorov-Smirnov test. The data were analyzed by two-way ANOVA ($p < 0.001$), followed by Tukey's test ($p < 0.05$) using the statistical Package for the Social Science software Program (version 10.0; SPSS).

Table 1
Primers used for quatitative PCR assay.

Adaptation			Sequence (5′-3′)	
qPCR primer	v1a1	Forward	CCAAACTCCGCACCGTCAA	
		Reverse	ATGCGGATAGGGTCACTGCT	(150 bp)
	v1a2	Forward	TAGTGTGCTGGGCACCGTT	
		Reverse	GATCCAGGGGTTGCAGCAG	(140 bp)
	v2a	Forward	CAGCGTGAGCACCATCTCC	
		Reverse	ATGCGGATAGGGTCACTGCT	(173 bp)
DNA Control	β-actin	Forward	TGGCCGTGACCTGACTGAC	
		Reverse	CCTGCTCAAAGTCAAGAGCGAC	(157 bp)

3. Results

3.1. In vivo effects of E_2

The E_2 treatment influenced only *v1a1* and *v1a2* expression in the brain and ovary, but not that of *v2a* (data not shown).

Brain – In the preparatory phase, the E_2 treatment produced an overall significant effect on the *v1a1* expression (Fig. 1A; two way ANOVA, $p < 0.001$, $F_{dose} = 110$, $F_{duration} = 134$, $F_{interaction} = 97.8$). The expression varied with the dose and duration. The lower dose of 0.05 μg E_2 elicited a significant increase in the transcript levels at 16 and 24 h ($p < 0.05$, Tukey's test). But in the median dose of 0.1 μg E_2, the *v1a1* expression was up regulated at all time points; the maximal response was reached as early as 8 h and then decreased significantly at 16 and 24 h compared to the 8 h stimulation. The highest dose of 0.5 μg E_2 did not alter the gene expression. In the prespawning phase, the E_2 treatment produced an overall significant effect on the expression of *v1a1* (Fig. 1B; two way ANOVA, $p < 0.001$, $F_{dose} = 29.64$, $F_{duration} = 62.28$, $F_{interaction} = 10.9$). The *v1a1* expression was inhibited by the E_2 treatment in comparison to the control levels and the inhibition varied with dose and duration ($p < 0.05$, Tukey's test).

The E_2 treatment elicited an overall significant effect on the expression of *v1a2* transcript levels in the preparatory phase (Fig. 1C; two way ANOVA, $p < 0.001$, $F_{dose} = 106.89$, $F_{duration} = 134.27$, $F_{interaction} = 79.64$), and in the prespawning phase (Fig. 1D; two way ANOVA, $p < 0.001$, $F_{dose} = 46.24$, $F_{duration} = 84.54$, $F_{interaction} = 18.92$). In the preparatory phase, the E_2 treatment up regulated the expression at 16 and 24 h in the 0.05 μg group and at 8 and 16 h in the 0.1 and 0.5 μg groups. In the prespawning phase, the steroid treatment inhibited the *v1a2* expression and the effect was greater at 16 and 24 h in the highest dose (0.5 μg) group.

Ovary – The E_2 administration produced an overall significant effect on the expression of *v1a1* in the preparatory (Fig. 2A; two way ANOVA, $p < 0.001$, $F_{dose} = 167$, $F_{duration} = 280$, $F_{interaction} = 171$) and prespawning (Fig. 2B; two way ANOVA, $p < 0.001$, $F_{dose} = 39.56$, $F_{duration} = 87.12$, $F_{interaction} = 13.43$) phases. In the preparatory phase, E_2 up regulated the transcript levels at all duration in the 0.05 μg group with the highest fold increase at 16 h. In the 0.1 μg group, a significant up regulation was observed at 8 h and 16 h with the highest fold increase at 8 h. At 24 h, the treatment failed to produce any significant effect. In the 0.5 μg group, the response was significant but low at 8 and 16 h, and insignificant at 24 h. In the prespawning phase, the E_2 treatment inhibited the transcript levels compared to the control groups.

The expression of *v1a2* showed an overall significant effect after the E_2 treatment in the preparatory (Fig. 2C; two way ANOVA, $p < 0.001$, $F_{dose} = 114$, $F_{duration} = 146$, $F_{interaction} = 45$), and in the prespawning (Fig. 2D; two way ANOVA, $p < 0.001$, $F_{dose} = 39.56$, $F_{duration} = 87.12$, $F_{interaction} = 13.43$) phases. In the preparatory phase, the steroid treatment up regulated the expression at 16 and 24 h in the 0.1 μg group and at 8 h in the 0.1 μg group. The highest dose of 0.5 μg E_2 did not elicit any significant effect on the expression. In the prespawning phase, the transcript levels were inhibited at all duration in the 0.05 and 0.1 μg groups, and at 16 and 24 h in the 0.5 μg group.

3.2. In vitro effects of E_2 on ovarian VT receptor gene expression

The incubation of the ovarian tissues with E_2 produced an overall significant effect on *v1a1* expression in the preparatory (Fig. 3A; two

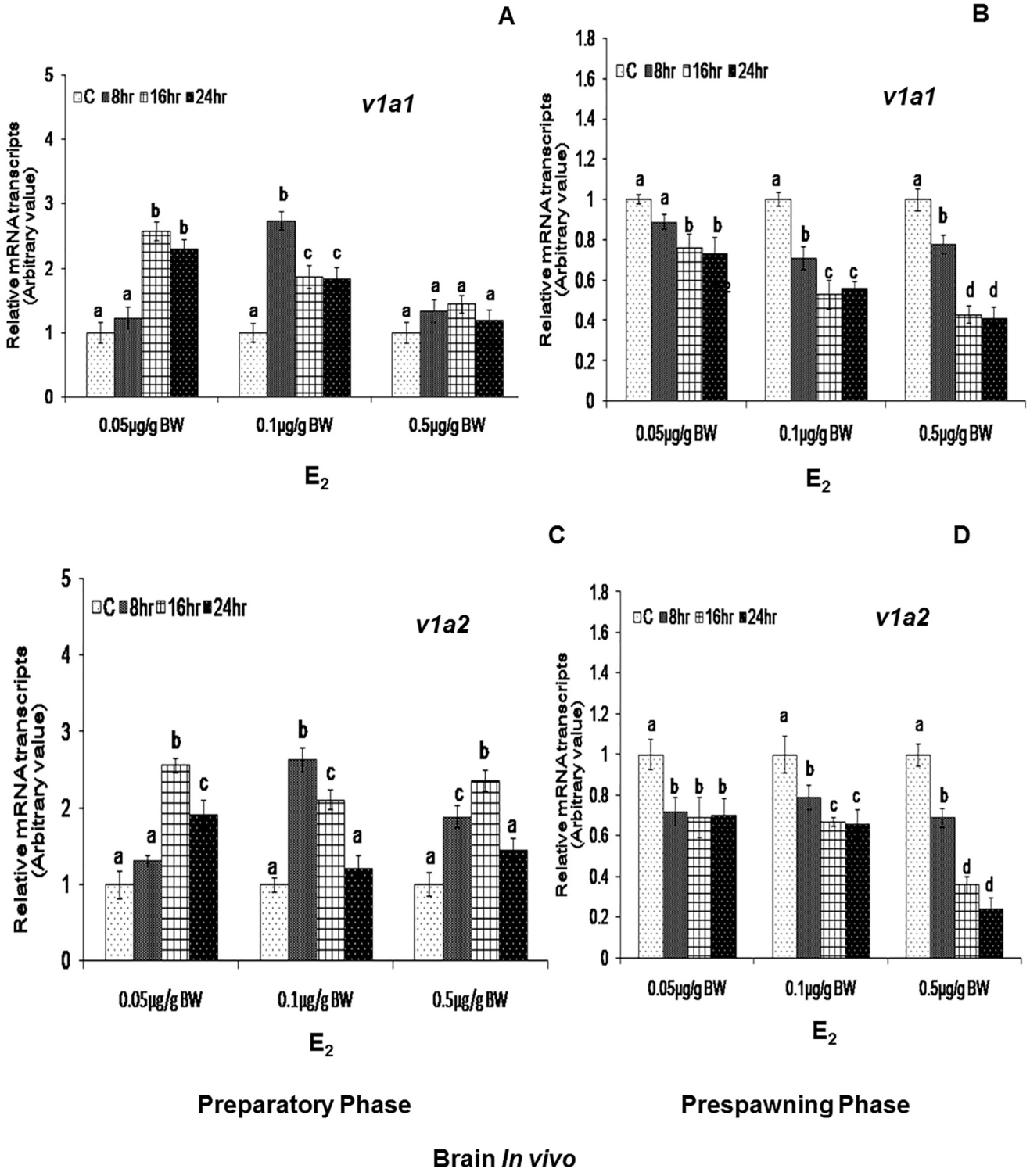

Fig. 1. *In vivo* effects of estradiol-17β (E_2) on brain *v1a1* (A, B) and *v1a2* (C, D) expression (mean ± SEM; n = 5) in the preparatory and prespawning phases, respectively. Groups bearing the same letters are not significantly different (two way ANOVA, $p < 0.001$, Tukey's test, $p < 0.05$).

way ANOVA, $p < 0.001$, $F_{dose} = 143.56$, $F_{duration} = 223.56$, $F_{interaction} = 131$) and prespawning (Fig. 3B; two way ANOVA, $p < 0.001$, $F_{dose} = 48.51$, $F_{duration} = 79.42$, $F_{interaction} = 15.12$) phases. In the preparatory phase, E_2 elicited a dose- related decrease in the expression with the highest fold increase at the lowest concentration of 1 nM group. The response varied with duration; the highest fold increase was at 16 h in 1 nM and 100 nM E_2, and at 8 h in the 10 nM group. At 24 h, a significant increase was noticed only in the 1 nM group. In the 100 nM group, the E_2 response was low and the increase was significant at 8 and 16 h. In the prespawning phase, E_2 inhibited the *v1a1* expression and the inhibition was low in the 100 nM group.

The expression of *v1a2* showed an overall significant effect after incubation with E_2 in the preparatory (Fig. 3C; two way ANOVA, $p < 0.001$, $F_{dose} = 110$, $F_{duration} = 145$, $F_{interaction} = 79$) and prespawning (Fig. 3D; two way ANOVA, $p < 0.001$, $F_{dose} = 32.16$, $F_{duration} = 83.32$, $F_{interaction} = 10.21$) phases. In the preparatory phase,

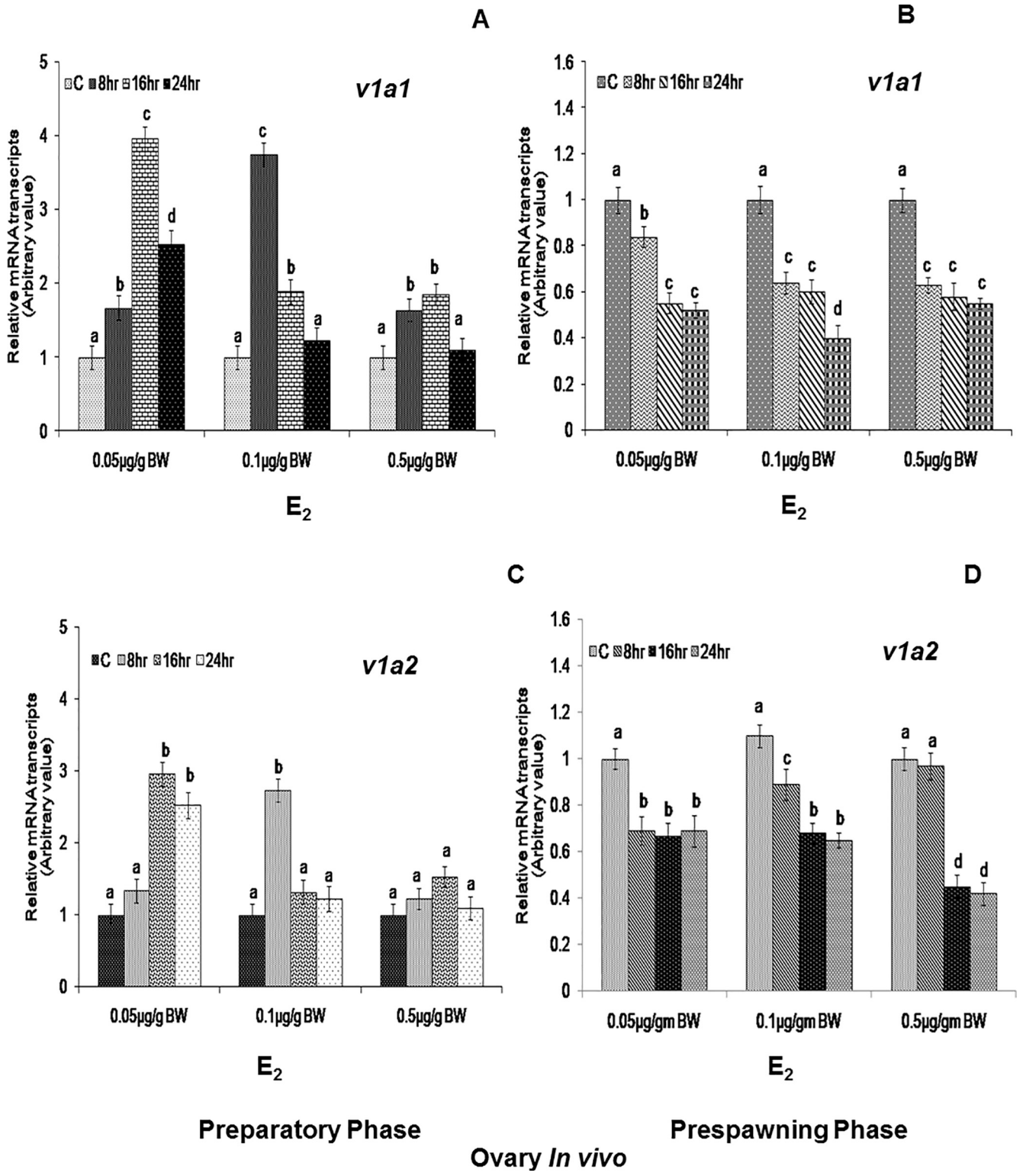

Fig. 2. *In vivo* effects of estradiol-17β (E_2) on ovarian *v1a1* (A, B) and *v1a2* (C, D) expression (mean ± SEM; n = 5) in the preparatory and prespawning phases, respectively. Groups bearing the same letters are not significantly different (two way ANOVA, $p < 0.001$, Tukey's test, $p < 0.05$).

the response was similar to that of the *v1a1* expression but with a low fold increase in the low dose groups. In the prespawning phase, E_2 inhibited the *v1a2* expression and the effect was stronger in the 100 nM group.

The incubation with E_2 did not alter the expression of *v2a* in the ovary in both preparatory and prespawning phases (data not shown).

3.3. *In vivo* effects of 17, 20β-DP

The 17, 20β-DP treatment influenced only *v1a1* and *v1a2* expression in the brain and ovary, but not that of *v2a* (data not shown).

Brain – The 17, 20β-DP treatment produced an overall significant effect on *v1a1* expression in the preparatory (Fig. 4A; two way ANOVA, $p < 0.001$, $F_{dose} = 41.70$, $F_{duration} = 95.28$, $F_{interaction} = 15.57$) and

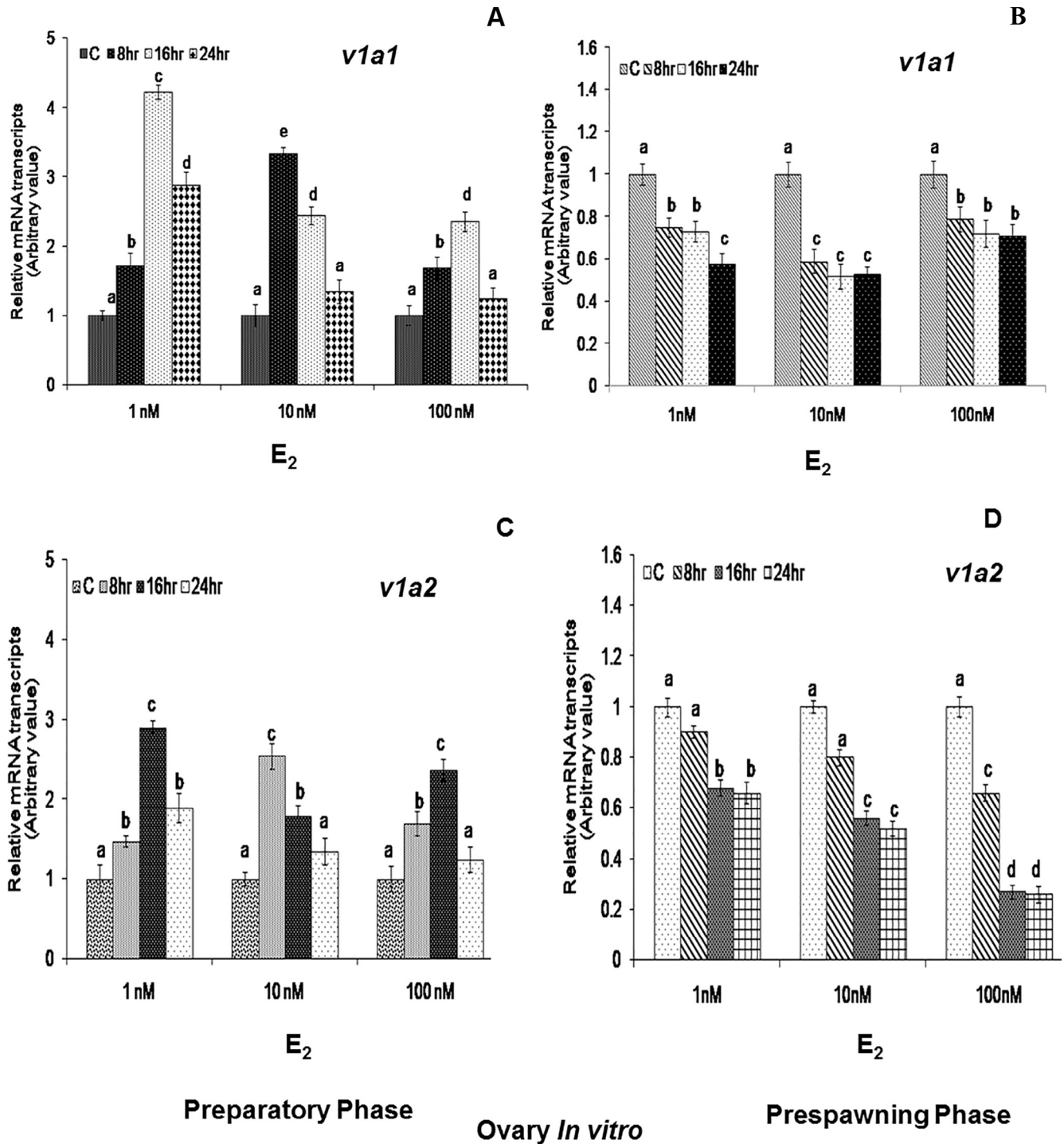

Fig. 3. *In vitro* effects of estradiol-17β (E_2) on the expression (mean ± SEM; n = 5) of *v1a1* (A, B) and *v1a2* (C, D) in the ovary pieces/ovarian follicles in the preparatory and prespawning phases, respectively. Groups bearing the same letters are not significantly different (two way ANOVA, $p < 0.001$, Tukey's test, $p < 0.05$).

prespawning (Fig. 4B; two way ANOVA, $p < 0.001$, $F_{dose} = 95.24$, $F_{duration} = 108.65$, $F_{interaction} = 39.48$) phases. In both phases, the lowest dose of 0.1 μg did not influence the transcript levels. In the preparatory phase, the fold increase was low and was significant at 16 and 24 h in both 0.5 and 1 μg groups. In the prespawning phase, the expression was up regulated at all time points in both 0.5 and 1 μg groups and the fold-increase was higher than that of the preparatory phase.

The steroid treatment produced an overall significant effect on *v1a2* expression in the preparatory (Fig. 4C; two way ANOVA, $p < 0.001$, $F_{dose} = 42.61$, $F_{duration} = 93.46$, $F_{interaction} = 18.55$) and prespawning (Fig. 4D; two way ANOVA, $p < 0.001$, $F_{dose} = 292.63$, $F_{duration} = 183.24$, $F_{interaction} = 98.17$) phases. In the preparatory phase, the *v1a2* expression was stimulated modestly; the stimulation was significantly high at 24 h in the 0.1 μg group and at 16 h and 24 h in the 0.5 and 1 μg groups. In the prespawning phase, the *v1a2* expression was significantly up regulated at 16 and 24 h in the 0.5 μg group and at all time points in the 1 μg group. The stimulation was the highest in this phase in comparison to that of the preparatory phase.

Ovary – The 17, 20β-DP treatment produced an overall significant

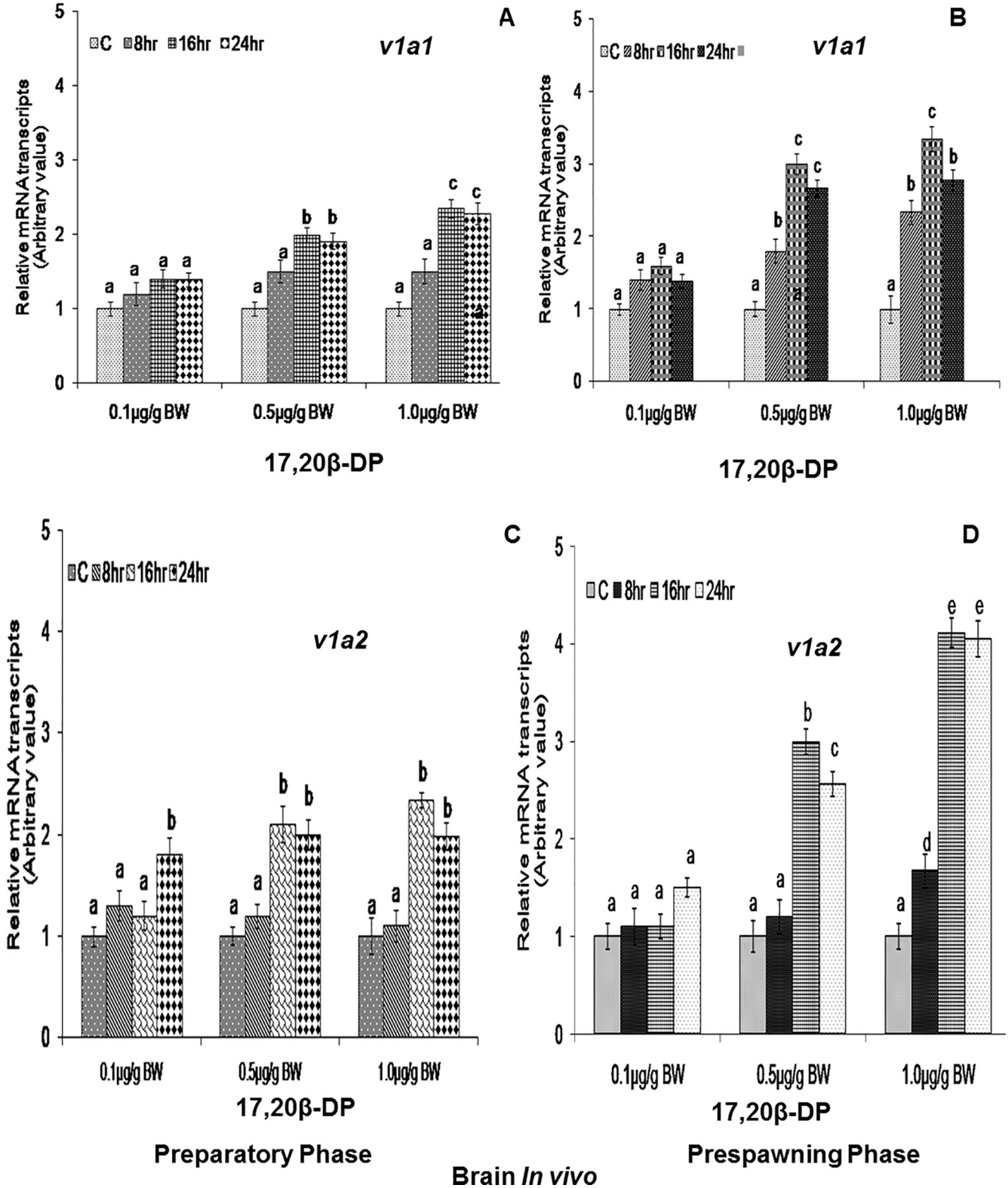

Fig. 4. *In vivo* effects of the maturation-inducing steroid 17, 20β-DP on brain *v1a1* (A, B) and *v1a2* (C, D) expression (mean ± SEM; n = 5) in the preparatory and prespawning phases, respectively. Groups that bear the same letters are not significantly different (two way ANOVA, $p < 0.001$, Tukey's test, $p < 0.05$).

effect on *v1a1* expression in the preparatory (Fig. 5A; two way ANOVA, $p < 0.001$, $F_{dose} = 17.65$, $F_{duration} = 40.43$, $F_{interaction} = 12.77$) and prespawning (Fig. 5B; two way ANOVA, $p < 0.001$, $F_{dose} = 90.93$, $F_{duration} = 132.53$, $F_{interaction} = 27.86$) phases. In the preparatory phase, the *v1a1* expression was stimulated only in the 0.5 µg group at 16 h. In the prespawning phase, the expression was up regulated both in the 0.5 and 1 µg groups at all time points with the highest fold increase at 16 h.

The 17, 20β-DP treatment produced an overall significant effect on *v1a2* expression in the preparatory (Fig. 5C; two way ANOVA, $p < 0.001$, $F_{dose} = 18.3$, $F_{duration} = 48.2$, $F_{interaction} = 14.18$) and prespawning (Fig. 5D; two way ANOVA, $p < 0.001$, $F_{dose} = 93.24$,

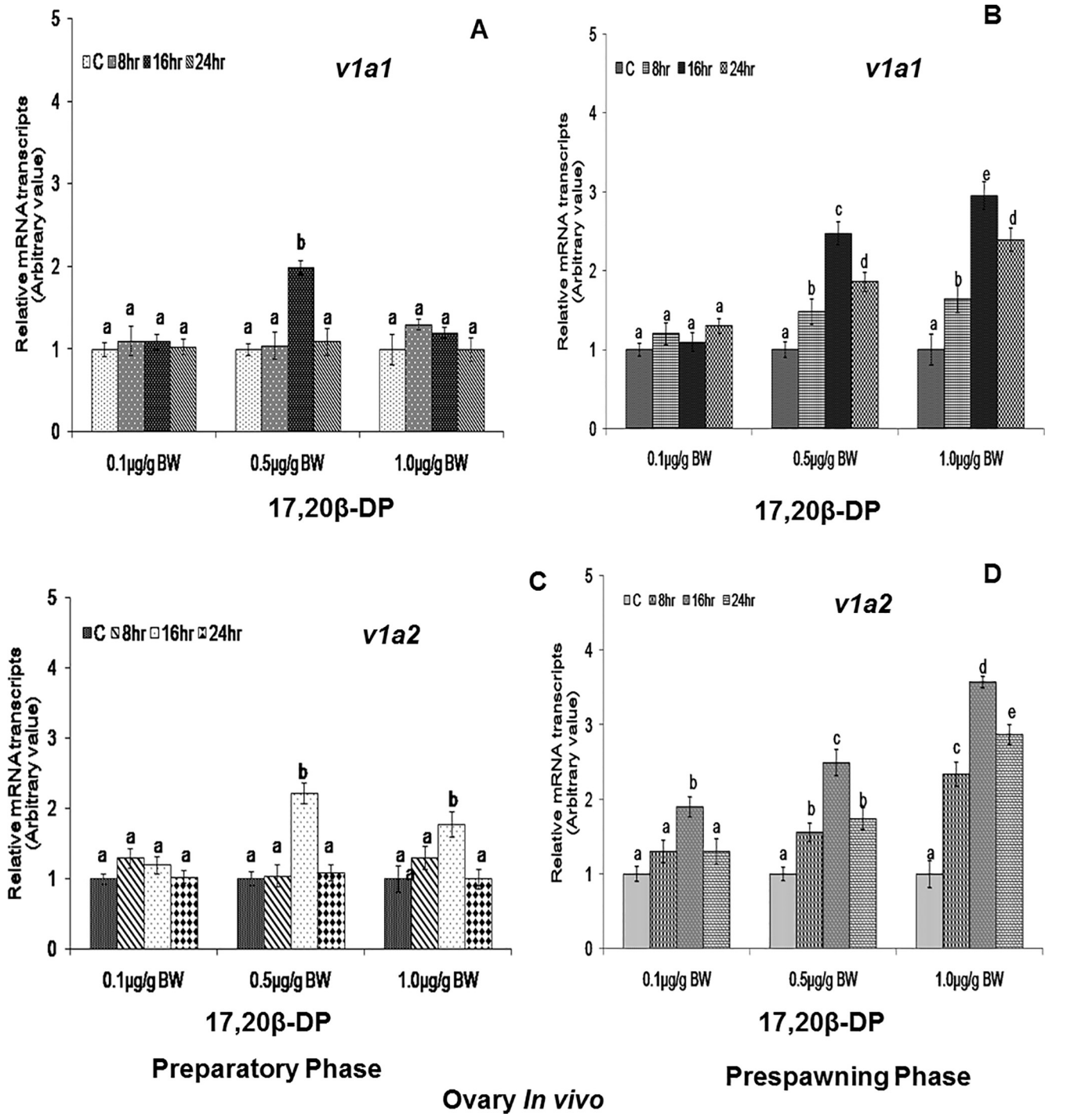

Fig. 5. *In vivo* effects of the maturation-inducing steroid 17, 20β-DP on ovarian *v1a1* (A, B) and *v1a2* (C, D) expression (mean ± SEM; n = 5) in the preparatory and prespawning phases, respectively. Groups that bear the same letters are not significantly different (two way ANOVA, $p < 0.001$, Tukey's test, $p < 0.05$).

$F_{duration} = 165.6$, $F_{interaction} = 25.67$) phases. In the preparatory phase, the *v1a2* expression was stimulated only at 16 h in the 0.5 and 1 µg groups. In the prespawning phase, the expression increased at 16 h in the 0.1 µg group and at all time points in the 0.5 and 1 µg groups. The expression was up regulated dose-dependently and was higher compared to the preparatory phase. The fold-increase was the highest at the 16 h time points in all dose groups.

3.4. In vitro effects of 17, 20β-DP

3.4.1. Ovarian VT receptor gene expression

In vitro incubation of ovarian tissues with 17, 20β-DP produced an overall significant effect on *v1a1* expression in the preparatory (Fig. 6A; two way ANOVA, $p < 0.001$, $F_{dose} = 38.34$, $F_{duration} = 78.6$, $F_{interaction} = 24.72$) and prespawning (Fig. 6B; two way ANOVA, $p < 0.001$, $F_{dose} = 193.24$, $F_{duration} = 125.6$, $F_{interaction} = 42.28$) phases. In the preparatory phase, the *v1a1* transcript level was not affected in the 1 nM and 100 nM groups. In the 10 nM group, the expression was strongly up regulated at 16 h and then declined at 24 h. In

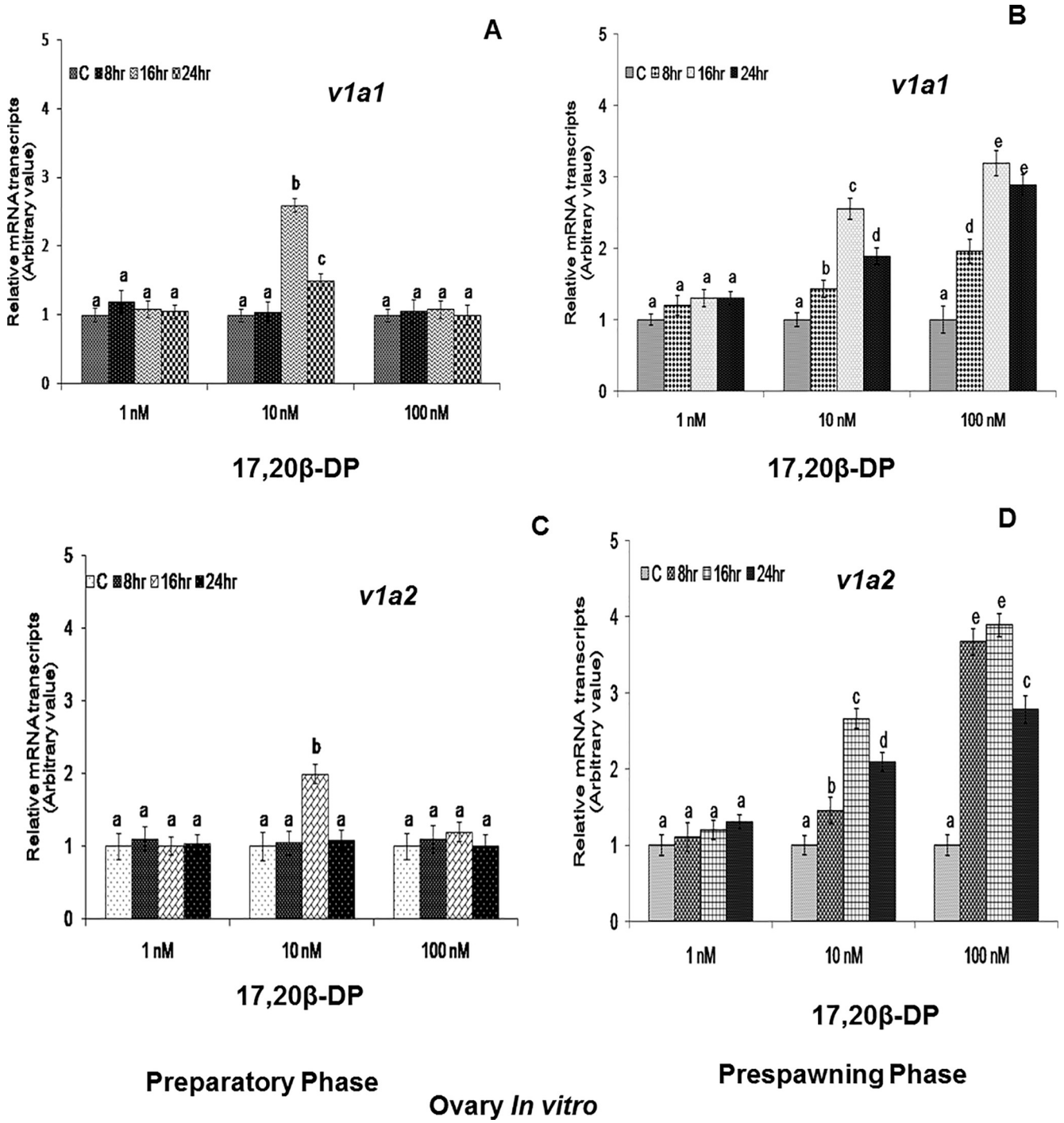

Fig. 6. *In vitro* effects of the maturation-inducing steroid 17, 20β-DP on the expression (mean ± SEM; n = 5) of *v1a1* (A, B) and *v1a2* (C, D) in ovary pieces/ovarian follicles in the preparatory and prespawning phases, respectively. Groups that bear the same letters are not significantly different (two way ANOVA, $p < 0.001$, Tukey's test, $p < 0.05$).

the prespawning phase, the *v1a1* expression was up regulated dose-dependently in the 10 nM and 100 nM groups with the highest fold increase at 16 h. There was no effect in the 1 nM group.

The 17, 20β-DP incubation has produced an overall significant effect on *v1a2* expression in the preparatory (Fig. 6C; two way ANOVA, $p < 0.001$, $F_{dose} = 33.14$, $F_{duration} = 65.6$, $F_{interaction} = 20.87$) and prespawning (Fig. 6D; two way ANOVA, $p < 0.001$, $F_{dose} = 218.15$, $F_{duration} = 140.26$, $F_{interaction} = 66.86$) phases. In the preparatory phase, the *v1a2* expression was stimulated only at 16 h in the 10 nM group. In the prespawning phase, the transcript levels were not altered in the 1 nM group and increased significantly in a dose-dependent manner in the 10 nM and 100 nM groups. The stimulation was the highest at 16 h in the 10 nM group, and at 8 h and 16 h in the 100 nM group.

The 17, 20β-DP treatment did not alter the expression of *v2a* transcripts in both preparatory and prespawning phases (data not shown).

3.4.2. GVBD

The incubation with 17, 20β-DP elicited an overall significant effect on GVBD (Table 2; $p < 0.001$, $F_{dose} = 10.23$ $F_{duration} = 15.78$ $F_{interaction} = 18.56$, two way ANOVA). The MIS-induced stimulation was concentration- and duration-dependent with a 50% score in the

Table 2
In vitro effects of 17, 20β-dihydroxy-4-pregnen-3-one (MIS) and human chorionic gonadotropin (hCG) on germinal vesicle breakdown (GVBD; means ± SEM; n = 150 follicles in each group) in the catfish *Heteropneustes fossilis*.

Incubations	GVBD in percentage			
MIS	0 h	8 h	16 h	24 h
1 nM	0	6 ± 0.005[a]	8 ± 0.002[a]	15 ± 0.04[b]
10 nM	0	10 ± 0.04[c]	24 ± 0.032[d]	32 ± 0.005[e]
100 nM	0	23 ± 0.015[d]	42 ± 0.057[f]	50 ± 0.035[g]
hCG				
20 IU	0	14 ± 0.037[a]	25 ± 0.048[b]	36 ± 0.028[c]
50 IU	0	28 ± 0.03[d]	39 ± 0.047[c]	66 ± 0.021[e]
100 IU	0	58 ± 0.055[f]	87 ± 0.025[g]	100 ± 0.022[h]

The data were analyzed by two way ANOVA ($P < 0.01$) and Tukey's test ($P < 0.05$). Groups bearing the same alphabets aa, and dd in the MIS experiment and cc in the hCG experiment are not significant and those bearing different alphabets a–g in each experiment are significant with each other.

100 nM group at 24 h.

3.5. In vivo effects of hCG

The hCG administration did not alter the expression of VT receptor genes in the preparatory phase (data not shown).

Brain – In the prespawning phase, the hCG treatment produced an overall significant effect on *v1a1* (Fig. 7A; two way ANOVA, $p < 0.001$, $F_{dose} = 293.88$, $F_{duration} = 215.16$, $F_{interaction} = 105.27$), *v1a2* (Fig. 7B; two way ANOVA, $p < 0.001$, $F_{dose} = 254.14$, $F_{duration} = 208.56$, $F_{interaction} = 95.39$) and *v2a* (Fig. 7C; two way ANOVA, $p < 0.001$, $F_{dose} = 93.24$, $F_{duration} = 65.62$, $F_{interaction} = 35.47$) expressions. The 20 IU of hCG did not produce any significant effect on the VT receptor gene transcript levels. The 50 IU hCG treatment increased the *v1a1* and *v1a2* expressions at 16 and 24 h. In the 100 IU hCG groups, both *v1a1* and *v1a2* expression was stimulated duration-dependently with the highest fold increase at 24 h. The *v2a* expression was unaltered in the 50 IU hCG group and was up regulated dose-dependently at 16 and 24 h in the 100 IU group.

Ovary – In the prespawning phase, the hCG treatment caused overall significant effects on *v1a1* (Fig. 8A; two way ANOVA, $p < 0.001$, $F_{dose} = 193.78$, $F_{duration} = 287.29$, $F_{interaction} = 93.46$), *v1a2* (Fig. 8B; two way ANOVA, $p < 0.001$, $F_{dose} = 184.21$, $F_{duration} = 284.73$, $F_{interaction} = 82.69$) and *v2a* (Fig. 8C; two way ANOVA, $p < 0.001$, $F_{dose} = 175.34$, $F_{duration} = 205.6$, $F_{interaction} = 66.94$) expressions. The 20 IU of hCG treatment did not alter the VT receptor gene transcript levels. The 50 IU also did not alter the transcript levels of *v2a*. The 50 IU hCG stimulated the *v1a1* expression duration-dependently. The 100 IU hCG treatment up regulated the expression at all duration but with a high fold increase at 16 h. The *v1a2* expression was stimulated by both 50 and 100 IU hCG with a high fold increase at 16 h. The *v2a* expression was significantly high at 16 h in the 100 IU group and the level dropped sharply at 24 h.

3.6. In vitro effects of hCG

3.6.1. Ovarian VT receptor gene expression

In the preparatory phase, the incubation of ovary pieces with hCG did not alter the expression of VT receptor genes in any group (data not shown). In the prespawning phase, the incubation of the ovarian tissues with hCG produced an overall significant effect on *v1a1* (Fig. 9A; two way ANOVA, $p < 0.001$, $F_{dose} = 184.36$, $F_{duration} = 295.47$, $F_{interaction} = 69.24$), *v1a2* (Fig. 9B; two way ANOVA, $p < 0.001$, $F_{dose} = 155.24$, $F_{duration} = 248.36$, $F_{interaction} = 59.19$) and *v2a* (Fig. 9C; two way ANOVA, $p < 0.001$, $F_{dose} = 175.34$, $F_{duration} = 205.6$, $F_{interaction} = 66.94$) expressions. The 20 IU of hCG treatment did not alter the VT receptor transcript levels. The *v1a1* expression was stimulated at all duration in the 50 and 100 IU hCG groups. The expression levels remained high at 16 and 24 h in the 50 IU group and decreased at 24 h in the 100 IU group. The *v1a2* expression was up regulated in both 50 and 100 IU hCG groups except at 8 h in the 50 IU group. The *v2a* expression was up regulated only at 24 h in the 50 IU group and both at 16 and 24 h in the 100 IU hCG group; the expression being the highest at 16 h.

3.6.2. Ovulation and GVBD

The hCG treatment *in vivo* resulted in ovulation of the fish according to the dose and duration. There was no ovulation in the 20 IU groups. In the 50 IU groups, one fish ovulated at 16 h and 2 fish at 24 h. In the 100 IU groups, the ovulation score was 3 fish at 8 h and all the fish at 16 h and 24 h. The incubation of ovarian follicles with hCG produced an overall significant effect on GVBD (Table 2; $P < 0.001$, $F_{dose} = 14.87$, $F_{duration} = 19.61$, $F_{interaction} = 26.08$, two way ANOVA). The effect was both concentration- and duration-dependent with 100% GVBD in the 100 IU group at 24 h.

4. Discussion

This study compliments the earlier study on the modulatory effects of the reproductive hormones on VT secretion in the brain and ovary of the catfish (Singh and Joy, 2009b) and draw parallelism with the pattern of changes obtained with steroid hormones and gonadotropin on the VT receptor gene expression and VT levels. The results show that while E_2, 17, 20β-DP and gonadotropin (hCG) modulate the expression of the v1a type receptor gene paralogs (*v1a1 and v1a2*), only the gonadotropin modulates the *v2a* transcript levels in both brain and ovary.

4.1. Steroid hormone regulation of VT receptor gene expression

In teleosts including the catfish, the principal ovarian steroids are E_2 that regulates ovarian growth (vitellogenesis) and the progesterone derivative 17, 20β-DP or 17, 20, 21β-trihydroxyprogesterone that acts as the MIS to reinitiate meiotic maturation and induce ovulation (Nagahama, 1987; Mishra and Joy, 2006). In the catfish, there is a gradual rise in the E_2 level in the ovary and plasma during the transformation of the previtellogenic follicles to vitellogenic follicles, and then a decline in the level occurs after the completion of vitellogenesis (post vitellogenic phase) with a concomitant increase in the level of progestin steroids (progesterone, 17-hydroxyprogesterone and 17, 20β-DP) (Mishra and Joy, 2006). Keeping the steroidogenic pattern in view, we compared the effects of the major steroids on brain and ovarian VT receptor gene paralogs in previtellogenic and post-vitellogenic stages. The present data show that E_2 modulated brain and ovarian *v1a1* and *v1a2* expression differently in the two stages: an overall stimulatory effect in the previtellogenic stage and an inhibitory effect in the post-vitellogenic stage. On the other hand, 17, 20β-DP is stimulatory in both phases but more strongly in the prespawning phase. Both steroids failed to evoke any response on *v2a* expression either *in vivo* or *in vitro*.

In the previtellogenic phase, *v1a1* and *v1a2* expression was up regulated by E_2 in the brain (*in vivo*) and ovary (*in vivo* and *in vitro*). The effect varied with the dose of E_2 and duration of the treatment. While in the *in vivo* study, the lower doses of E_2 (0.05 and 0.1 μg) were almost equally effective to stimulate both *v1a1* and *v1a2* transcript levels in the brain and ovary, the high dose (0.5 μg) was ineffective or produced a low stimulation of the transcripts. Under *in vitro* conditions, an inverse trend in E_2 concentration versus expression profile was noticed: the lowest concentration of E_2 (1 nM) produced the highest fold-increase in ovarian *v1a1* and *v1a2* expression and the expression levels decreased with the increase in the dose. There was also a time shift in the appearance of the fold-increase: 16 h in 1 nM and 100 nM concentrations and 8 h in the 10 nM concentration. In the post vitellogenic ovary, E_2 *in vivo* or *in vitro* produced an inhibitory effect on the expression of both *v1a1* and *v1a2* in the brain and ovary. The stage-specific differential

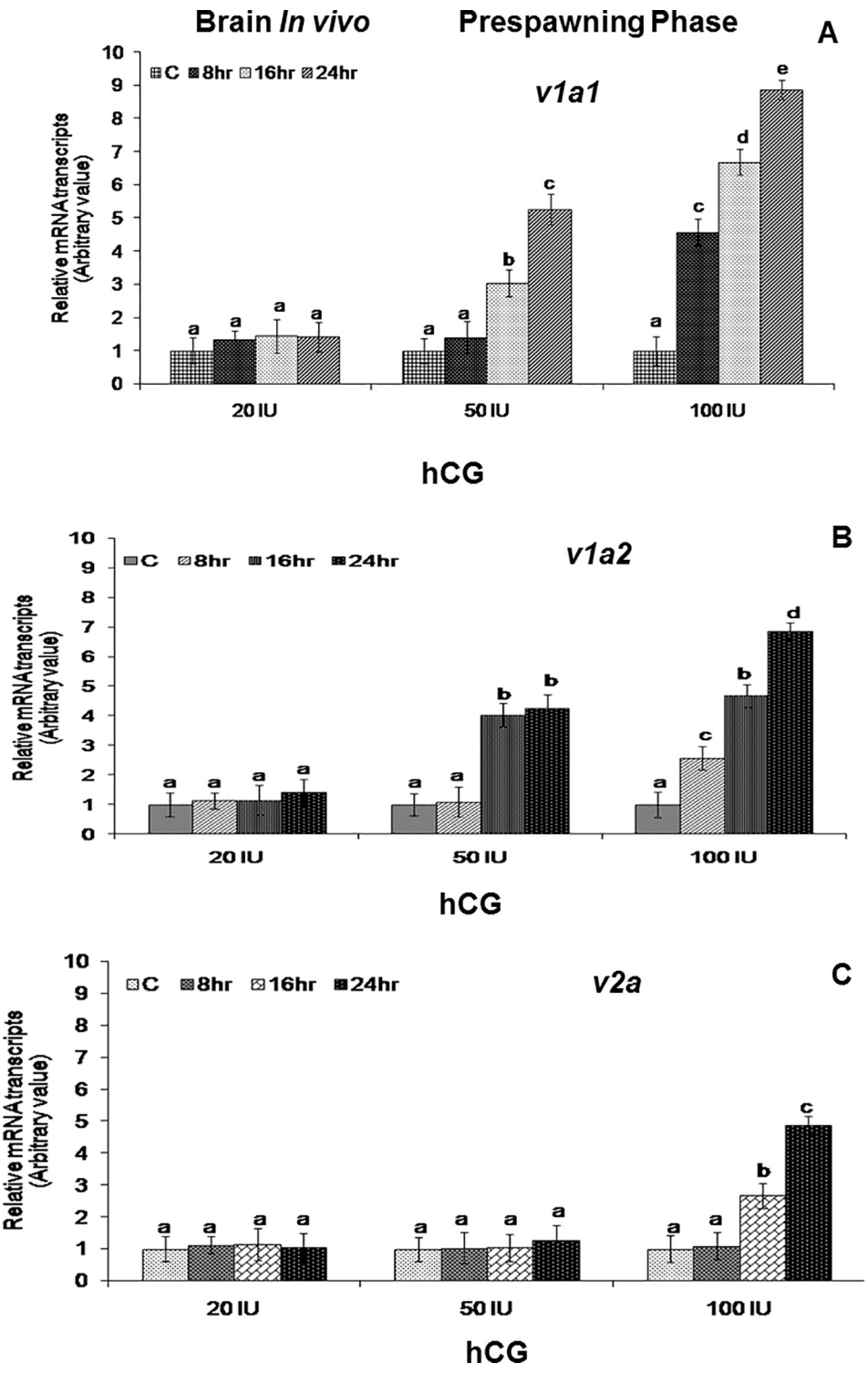

Fig. 7. *In vivo* effects of human chorionic gonadotropin (hCG) on brain *v1a1* (A), *v1a2* (B) and *v2a* (C) expression (mean ± SEM; n = 5) in the prespawning phase. Groups that bear the same letters are not significantly different (two way ANOVA, $p < 0.001$, Tukey's test, $p < 0.05$).

effects can be attributed to tissue sensitivity to the steroid or its titer. In ovariectomized and E_2 replaced catfish, E_2 elicited a dose-related response on *v1a* transcript levels; only the lower dose restored the ovariectomy-induced inhibition (Rawat et al., 2016). Similar changes in the levels of the receptor agonist VT were also noted after ovariectomy and E_2 replacement. In 3-week ovariectomized catfish, a low dose (0.1 μg) of E_2 compensated the ovariectomy-induced decrease in the VT levels while a high dose (0.5 μg) inhibited the levels (Singh and Joy, 2009b). In the same study, it was reported that E_2 elicited biphasic effects on VT levels depending on the season and maturity of the follicles, *in vitro*. In the preparatory phase, E_2 produced a graded response on ovarian VT levels: at 16 h incubation, the low dose of 1 ng/mL stimulated the VT level maximally, while high doses of 10 ng/mL and 100 ng/mL) decreased the magnitude of the stimulation. On the other hand, in the prespawning phase, E_2 invariably inhibited the VT levels. There is a similarity in the overall E_2 modulation of the *v1a* receptor gene expression in the brain and ovary, in spite of the differences in dose and duration effects. This can be attributed to a direct autocine/paracrine

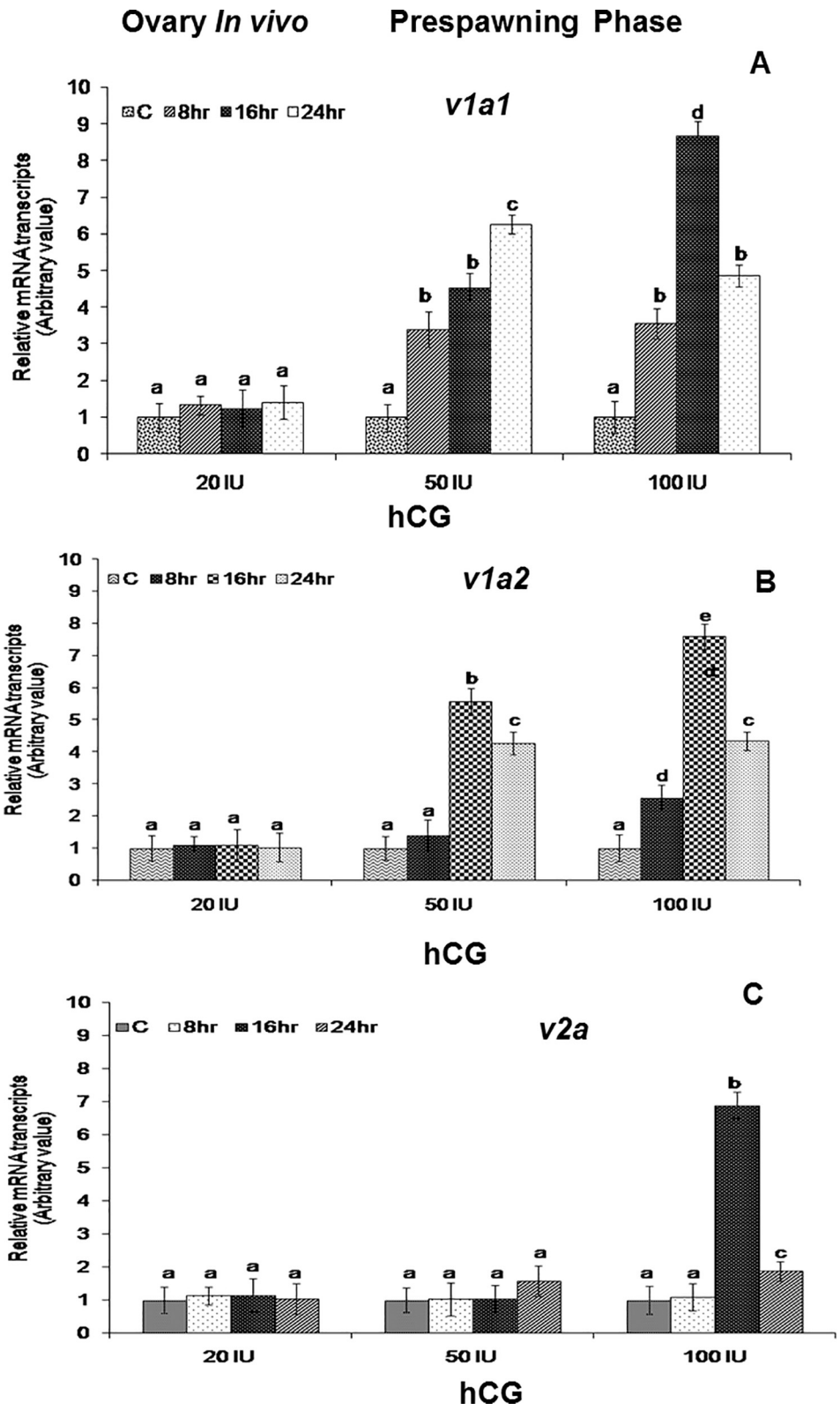

Fig. 8. *In vivo* effects of human chorionic gonadotropin (hCG) on ovarian *v1a1* (A), *v1a2* (B) and *v2a* (C) expression phases (mean ± SEM; n = 5) in the prespawning phase. Groups that bear the same letters are not significantly different (two way ANOVA, $p < 0.001$, Tukey's test, $p < 0.05$).

action of the steroid on the ovarian transcript levels (both E_2 and VT systems are localized in the follicular layer) and an indirect feedback control on the brain transcript levels. Taken together, it is summarized that VT and v1a receptor systems are sensitive to E_2 in the pre-vitellogenic stage (estrogenic phase) and insensitive to the steroid in the post-vitellogenic stage when the steroid biosynthesis and actions are at nadir and 17, 20β-DP is the major steroid (progesterone phase).

Unlike E_2, 17, 20β-DP stimulated both *v1a1* and *v1a2* transcript levels in the brain and ovary under *in vivo* or *in vitro* conditions. However, the response showed significant seasonal and tissue

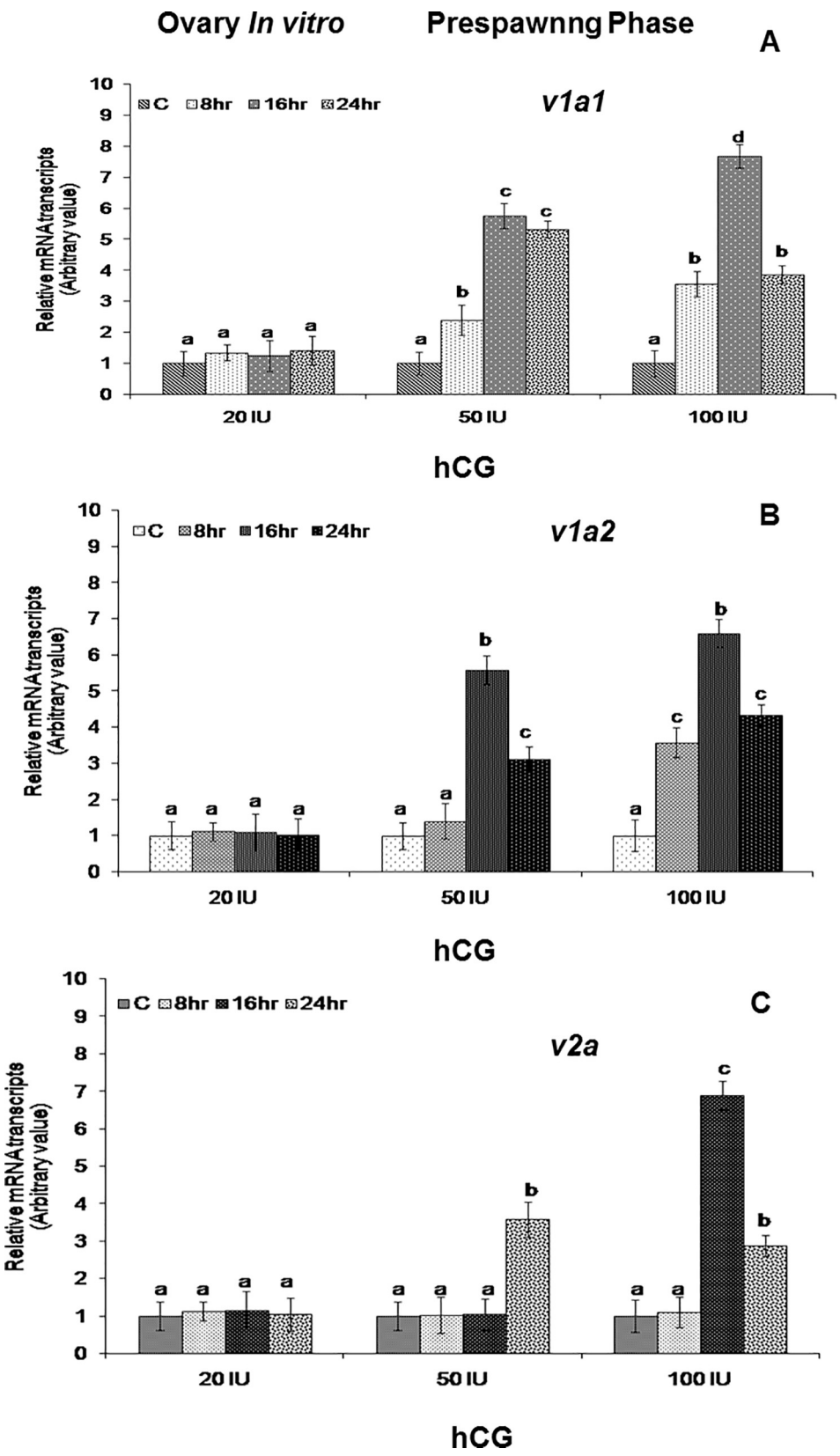

Fig. 9. *In vitro* effects of human chorionic gonadotropin (hCG) on *v1a1* (A), *v1a2* (B) and *v2a* (C) expression (mean ± SEM; n = 5) in ovary pieces/ovarian follicles in the prespawning phase. Groups that bear the same letters are not significantly different (two way ANOVA, $p < 0.001$, Tukey's test, $p < 0.05$).

variations. The 0.1 μg dose was generally ineffective in both seasons except for low stimulation in some groups. The high doses (0.5 μg and 1 μg) up regulated brain *v1a1* and *v1a2* expression at 16 h and 24 h, more strongly in the prespawning phase. In the ovary, the response was low and less pronounced in the preparatory phase but highly significant in the prespawning phase. More or less, a similar pattern was observed in the ovary *in vitro*. The relatively poor response in the previtellogenic ovary may be due to the fact that the follicular tissue may not be responsive to 17, 20β-DP. In this phase, the MIS is not synthesized or present in very small amounts in the catfish (Mishra and Joy, 2006). It was reported that both progesterone (P_4) and 17, 20β-DP increased VT levels in the post vitellogenic ovary or in isolated follicular layers with a higher effect by 17, 20β-DP (Singh and Joy, 2009b). Since the MIS induced GVBD, the v1a type receptor expression is associated with meiotic maturation and VT action in this process.

From the data, it is apparent that there is a differential regulation of VT and its receptors by steroids. E_2 is the major ovarian steroid in the preparatory phase and has a principal control on the VT system and 17, 20β-DP is the major steroid in the prespawning phase and takes over the role of E_2. Since both steroids stimulate VT secretion, it is likely that the ligand (VT) may directly up regulate its cognate receptors for functional readiness in which case the steroids may modulate the receptor expression indirectly through VT. The relatively high response in the brain *v1a* receptor gene expression in the prespawning phase may be due to the MIS feedback on the brain or indirectly via the MIS action on VT secretion. It is to be noted that the steroids has modulated only the *v1a* receptor gene expression since this receptor mediates the reproductive effects of VT (Joy and Chaube, 2015). On the contrary, unlike the *v1a* receptor gene modulation, the steroids have no role in the modulation of *v2a* receptor expression either in the preparatory phase or in the prespawning phase. It appears that this receptor subtype has a different control mechanism since it is not primarily concerned with reproductive functions (see below).

4.2. Gonadotropin control of VT receptor gene expression

Human CG has been used extensively to induce breeding in fishes including the catfish (Joy and Tharakan, 1999). In the present study also, hCG induced ovulation *in vivo* and GVBD *in vitro*. All the fish ovulated in the 100 IU groups at 16 h or 24 h. GVBD was scored 100% in the 100 IU groups at 24 h. In the catfish, hCG induced periovulatory changes in ovarian VT secretion with the peak increase at 16 h when the fish can be manually stripped to release eggs (Singh and Joy, 2009b). In contrast to the steroid hormone action, hCG up regulated the expression of all the three VT receptor genes in the prespawning phase. The lack of any response in the preparatory phase may be due to relatively low dose used (single treatment up to 100 IU) or the short duration study (up to 24 h). The low dose and short duration could not have sensitized the tissues to augment steroid production and the consequent action in the brain or ovary. The hCG effect in the prespawning phase can be related to the activation of the VT system directly or indirectly through the production of the MIS (Singh and Joy, 2009b). The highest fold-increase of both *v1a1* and *v1a2* at 24 h in the brain (50 and 100 IU groups) and at 16 h in the ovary (100 IU group) coincides with ovulatory events 16 h or 24 h. The high fold-increase in the expression of *v2a* was at 24 h in the brain and at 16 h in the ovary also coincides with ovulation. While the stimulatory effect of hCG on the *v1a1* and *v1a2* expression can be related to the MIS production and action (Mishra and Joy 2006), the hCG-induced stimulation of *v2a* may be due to a different mechanism (see below).

4.3. Functional significance: Role of VT system in final oocyte maturation and ovulation

In mammals, the control of reproductive and osmoregulatory functions vasopressin (VP) and oxytocin (OT) is mediated through specific receptors linked to distinct cell signalling pathways (V2 receptor to cAMP-PKA pathway and OTR through PLC-PKC pathway) (Banerjee et al., 2017). VT performs both the functions of VP and OT through the mediation of distinct receptors: V1a type and V2 type receptors. The V1a type receptor is linked to the PLC-PKC pathway like OTR while the V2 receptor is linked to the cAMP-PKA pathway. The results show that steroid hormones modulate only the oxytocic (reproductive) functions of VT through the v1a type receptors and not the antiduretic/diuretic actions of VT through the v2a receptor. The estrogen modulation of v1a type receptors in the catfish represents a phylogenetically well conserved mechanism in vertebrates (Larcher et al., 1995; Fleming et al., 2006; Srivastava et al. 2008). Similarly, progesterone stimulation of oxytocic functions in mammals and *v1a* receptor gene expression in the catfish may represent a similar conserved mechanism of regulation. Physiological studies in the catfish showed that the VT- mediated ovarian steroidogenesis, MIS production, germinal vesicle break down and prostaglandin secretion are inhibited by a specific mammalian V1 type receptor antagonist (Singh and Joy, 2011; Joy and Singh, 2013), suggesting the involvement of the v1 type receptors. Since catfish has at least two v1 receptor gene paralogs, the VT actions can be mediated by either of the two or both. The strong E_2 control of VT and the v1 type receptor functions in the preparatory phase may be related to vitellogenesis and associated metabolic activity. VT's direct role on this function needs to be explored further. The steroid hormone regulation of v1 type receptor is thus directly related to reproductive functions of VT.

Since neither E_2 nor 17, 20β-DP elicits any role in the *v2a* expression, the hCG up regulation of the *v2a* receptor gene expression may not involve the steroids. Previously, we have reported a prominent role of VT in follicular hydration associated with meiotic maturation in the catfish (Singh and Joy, 2010). Aquaporins are involved in the hydration process and in the catfish a distinct ovarian aquaporin gene (*aquaporin1ab*) is characterized, which is also expressed in the brain (Chaube et al. 2011). The *aquaporin1ab* transcript levels showed seasonal variations with high expression in the prespawning-spawning phase. Both hCG and VT stimulated the expression of *aquaporin1ab*, VT at 6 h and hCG at 16 h (Acharjee et al. 2011). The MIS has a mild role in the expression of the *aquaporin* mRNA. The VT-induced *aquaporin1ab* expression was blocked by a specific mammalian V2 receptor blocker, and not by the V1 type receptor blocker. Since hCG stimulates periovulatory changes in VT secretion in the brain and ovary, the high levels of VT may be responsible for the stimulation of v2a receptor and induction of aquaporin1ab protein, purportedly to regulate water intake and movement in the brain and follicular hydration in the ovary. Therefore, the hCG-induced stimulation of *v2a* expression may be indirect through the ligand (VT) action. The activation of the VT system is functionally linked to the MIS- induced GVBD or the hCG-induced GVBD and ovulation in the catfish.

5. Conclusion

This study presents new insights into the hormonal regulation of VT receptor gene expression. The ovarian steroid hormones modulate the

Table 3
Summary of changes during *in vivo* or *in vitro* treatments with the hormones on the expression of vasotocin receptor mRNAs in the brain and ovary of the catfish *Heteropneustes fossilis*.

	Expression	*v1a1*	*v1a1*	*v1a2*	*v1a2*	*v2a*	*v2a*
Treatment	Season	PR	PSP	PR	PSP	PR	PSP
E_2		▲▼	▼	▲▼	▼	(−)	(−)
17, 20β-DP		▲	▲▲	▲	▲▲	(−)	(−)
hCG		(−)	▲	(−)	▲	(−)	▲

▲ – up regulation; ▼ – down regulation; ▲▼ – biphasic effect; (−) – no effect; PR – Preparatory phase; PSP – Prespawning phase.

v1a type receptor gene expression while gonadotropin (hCG) stimulates both *v1a* and *v2a* receptor gene expression (Table 3). While the steroid hormone modulation is found in both preparatory and prespawning phases, the hCG modulation seems to be restricted to the prespawning phase. E_2 stimulates *v1a* receptor expression in the previtellogenic ovary and inhibits it in the post vitellogenic stage. 17, 20β-DP stimulates *v1a* expression in both preparatory and prespawning phases but the magnitude of the up regulation is higher in the latter phase. Thus VT mediates diverse functions through differential and reproductive stage-specific modulation of its cognate receptors and the coupled cell signaling mechanisms.

Acknowledgments

This work was supported by a research grant of Department of Science and Technology, New Delhi (Grant No. SA/SO/AS-43/2009), to K. P. Joy (Principal Investigator) and R. Chaube (Co-Investigator). Dr. K. P. Joy is INSA Senior Scientist at Department of Biotechnology, Cochin University of Science and Technology, Kochi.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ygcen.2018.06.018.

References

Acharjee, A., Chaube, R., Joy, K.P., Cerda, J., 2011. Hormonal regulation of aquaporin-1ab in *Heteropneustes fossilis* oocytes *in vitro*. Indian J. Sci. Technol. 4, 165–166.

Acher, R., 1996. Molecular evolution of fish neurohypophysial hormones: neutral and selective evolutionary mechanisms. Gen. Comp. Endocrinol. 102, 157–172.

Amer, S., Brown, J.A, 1995. Glomerular actions of arginine vasotocin in the in situ perfused trout kidney. Am. J. Physiol. Regul. Integr. Comp. Physiol. 269 (4), 775–780.

Balment, R.J., Warne, J.M., 2006. Arginine vasotocin a key hormone in fish physiology and behavior: a review with insights from mammalian models. Gen. Comp. Endocrinol. 147, 9–16.

Banerjee, P., Joy, K.P., Chaube, R., 2017. Structural and functional diversity of non-apeptide hormones from an evolutionary perspective: a review. Gen. Comp. Endocrinol. 241, 4–23.

Chaube, R., Chauvigne, F., Sequeira, T.A., Joy, K.P., Acharjee, A., Singh, V., Cerda, J., 2011. Molecular and functional characterization of catfish (*Heteropneustes fossilis*) aquaporin- 1b: changes in expression during ovarian development and hormone-induced follicular maturation. Gen. Comp. Endocrinol. 170, 162–171.

Fleming, G.W., Spencer, T.E., Safe, S.H., Bazer, F.W., 2006. Estrogen regulates transcription of the ovine oxytocin receptor gene through GC rich SP1promoter elements. Endocrinology 14, 899–911.

Gimpl, G., Fahrenholz, F., 2001. The oxytocin receptor system: structure, function, and regulation. Physiol. Rev. 81, 629–683.

Goodson, J.L., Bass, A., 2000. Vasotocin innervation and modulation of vocal-acoustic circuitry in the teleost (*Porichthys notatus*). J. Comp. Neurol. 442, 363–379.

Joy, K.P., Chaube, R., 2015. Vasotocin-. A new player in the control of oocyte maturation and ovulation in fish. Gen. Comp. Endocrinol. 221, 54–63.

Joy, K.P., Singh, V., 2013. Functional interactions between vasotocin and prostaglandins during final oocyte maturation and ovulation in the catfish *Heteropneustes fossilis*. Gen. Comp. Endocrinol. 186, 126–135.

Joy, K.P., Tharakan, B., 1999. Induced spawning of the Indian catfish, *Heteropneustes fossilis*, by GnRH analogue alone or in combination with dopamine-affecting drugs. J. Appl. Aquacult. 9, 23–32.

Lagman, D., Ocampo Daza, D., Widmark, J., Abalo, X.M., Sundstro¨m, G., Larhammar, D., 2013. The vertebrate ancestral repertoire of visual opsins, transducin alpha subunits and oxytocin/ vasopressin receptors was established by duplication of their shared genomic region in the two rounds of early vertebrate genome duplications. BMC Evo. Biol. 13, 238.

Larcher, A., Neculcea, J., Breton, C., Arslan, A., Rozen, F., Russo, C., Zinggh, H., 1995. Oxytocin receptor gene expression in the rat uterus during pregnancy and the estrous cycle and in response to gonadal steroid treatment. Endocrinology 136, 5350–5356.

Lema, S.C., 2010. Identification of multiple vasotocin receptor cDNAs in teleost fish: sequences, phylogenetic analysis, sites of expression, and regulation in the hypothalamus and gill in response to hyperosmotic challenge. Mol. CellEndocrinol. 321, 215–230.

Lema, S.C., Slane, M.A., Salvesen, K.E., Godwin, J., 2012. Variation in gene transcript profiles of two V1a-type arginine vasotocin receptors among sexual phases of bluehead wrasse (*Thalassoma bifasciatum*). Gen. Comp. Endocrinol. 179, 451–464.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-DDCT method. Methods 25, 402–408.

Mishra, A., Joy, K.P., 2006. Effects of gonadotropin *in vivo* and 2-hydroxyestradiol- 17β *in vitro* on follicular steroid hormone profile associated with oocyte maturation in the catfish *Heteropneustes fossilis*. J. Endocrinol. 189, 341–353.

Ocampo Daza, D., Lewicka, M., Larhammar, D., 2012. The oxytocin/vasopressin receptor family has at least five members in the gnathostome lineage, including two distinct V2 subtypes. Gen. Comp. Endocrinol. 175, 135–143.

Nagahama, Y., 1987. 17a, 20β-dihydroxy-4-pregnen-3-one: a teleost maturation inducing hormone. Dev. Growth Differ. 29, 1–12.

Rawat, A., Chaube, R., Joy, K.P., 2015. Molecular cloning, sequencing and phylogeny of vasotocin receptor genes in the air-breathing catfish *Heteropneustes fossilis* with sex dimorphic and seasonal variations in tissue expression. Fish Physiol. Biochem. 41, 509–532. http://dx.doi.org/10.1007/s10695-015-0026-0.

Rawat, A., Chaube, R., Joy, K.P., 2016. Effects of ovariectomy and estrogen replacement on expression of brain vasotocin receptor subtype genes in the catfish *Heteropneustes fossilis*. J. Transl. Neurosci. 1, 1–4.

Salek, S.J., Sullivan, C.V., Godwin, J., 2002. Arginine vasotocin effects on courtship behaviour in make white perch (*Morone Americana*). Behav. Brain Res. 133, 177–1832.

Semsar, K., Godwin, J., 2003. Social influences on the arginine vasotocin system are independent of gonads in a sex-changing fish. J. Neurosci. 23, 4386–4393.

Singh, V., Joy, K.P., 2008. Immunocytochemical localization, HPLC characterization, and seasonal dynamics of vasotocin in the brain, blood plasma and gonads of the catfish *Heteropneustes fossilis*. Gen. Comp. Endocrinol. 159, 214–225.

Singh, V., Joy, K.P., 2009a. Relative *in vitro* seasonal effects of vasotocin and isotocin on ovarian steroid hormone levels in the catfish *Heteropneustes fossilis*. Gen. Comp. Endocrinol. 162, 257–264.

Singh, V., Joy, K.P., 2009b. Effects of hCG and ovarian steroid hormones on vasotocin levels in the female catfish *Heteropneustes fossilis*. Gen. Comp. Endocrinol. 162, 172–178.

Singh, V., Joy, K.P., 2010. An involvement of vasotocin in oocyte hydration in the catfish *Heteropneustes fossilis*: A comparison with effects of isotocin and hCG. Gen. Comp. Endocrinol. 166, 504–512.

Singh, V., Joy, K.P., 2011. Vasotocin induces final oocyte maturation and ovulation through production of a maturation-inducing steroid in the catfish *Heteropneustes fossilis*. Gen. Comp. Endocrinol. 174, 15–21.

Srivastava, R., Cornett, L.E., Chaturvedi, C.M., 2008. Effect of estrogen and its antagonist on the expression of arginine vasotocin (AVT) and its oxytocic-like receptor VT3 in the shell gland of Japanese quail, *Coturnix coturnix japonica*. Comp. Biochem. Physiol. A 151, 551–559.

Yamaguchi, Y., Kaiya, H., Konno, N., Iwata, E., Miyazato, M., Uchiyama, M., Bell, J.D., Toop, T., Donald, J.A., Venkatesh, B., Hyodo, S., 2012. The fifth neurohypophysial hormone receptor is structurally related to the V2-type receptor but functionally similar to V1-type receptors. Gen. Comp. Endocrinol. 178, 519–528.

Wells, A., Anderson, W.G., Hazon, N., 2002. Development of an *in situ* perfused kidney preparation for elasmobranch fish: action of arginine vasotocin. Am. J. Physiol. 282, R1636–R1642.