Endocr (2007) 31:154–160
DOI 10.1007/s12020-007-0023-x

ORIGINAL PAPER

Modulation of cardiac oxytocin receptor and estrogen receptor alpha mRNAs expression following neonatal oxytocin treatment

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Received: 23 February 2007 / Accepted: 2 May 2007 / Published online: 13 June 2007

Abstract Oxytocin (OT) is known for its role in reproduction. However, evidence has emerged suggesting its involvement in the regulation of the cardiovascular system. Here we examine the hypothesis that neonatal exposure to OT can have both short-term and long-lasting consequences on gene expression in heart tissue. On the first day of postnatal life, female and male prairie voles (*Microtus ochrogaster*) were randomly assigned to receive one of following treatments: 50 µl i.p. injection of (a) 3 µg OT (b) 0.3 µg of OT antagonist (OTA), or (c) isotonic saline (SAL). Hearts were collected on postnatal day 1 (D1, 2 h after injection), day 8 (D8), or day 21 (D21), and the mRNA expression for OT receptor (OTR), estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ as a function of age, treatment, and sex were measured using RT-PCR. Neonatal treatment with OT showed a marked increase in cardiac OTR mRNA expression on postnatal D1, but not D8 or D21, in both female and male animals. ERα increased as a function of OT treatment only in females. Although significant treatment effects were no longer detected in D8 or D21 animals, there were significant changes in the relative expression of all types of mRNA between D1 and D21 with age-related declines in OTR and ERβ and increases in ERα Neonatal treatment with OTA showed no changes in cardiac OTR, ERα, or ERβ mRNAs expression. The results indicate that during the early postnatal period OT can have rapid effects on the expression of OTR and ERα mRNAs and that these effects are mitigated by D8 or D21. Also, with the exception of ERα mRNA, the effects are the same in both sexes.

Keywords Heart · Oxytocin · Oxytocin receptor · Estrogen receptor

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Introduction

\Oxytocin (OT), a nanopeptide hormone, is synthesized primarily in the hypothalamus [1]. It is also produced in peripheral tissues [2], including heart [3]. In addition to OT effects on reproductive functions and induction of maternal behavior [4, 5], it is involved in endocrine and neuroendocrine regulation of heart, vasculature, and kidney [6–9]. OT perfusion of isolated rat hearts results in a dose-dependent negative chronotropic effect while exerting a positive inotropic effect [10]. It has been reported that OT acts via neuroendocrine-endocrine-paracrine pathways to regulate blood volume via its natriuretic properties and to modulate blood pressure by stimulating the release of atrial natriuretic peptide [6, 9]. Furthermore, OT stimulates the release of nitric oxide (NO) from human umbilical vein endothelial cells in culture [7]. These findings have led to the suggestion that OT may be an important mediator of cardiovascular function. Empirical studies suggest that OT may affect or regulate the function of the heart through several different mechanisms.

Estrogen mediates gene transcription through the activation of two receptors [11]: estrogen receptor alpha and

beta (ERα and ERβ). Recent observations suggest that estrogen effects in vascular cells, and possibly in the myocardium, depend on the relative expression of ERα and ERβ [12]. Interactions between OT and estrogen have been reported in several systems. In MCF7 breast cancer cell lines treated with OT inhibited the ability of estrogen to stimulate mitosis [13]. The OT treatment had a number of effects on ERα in the MCF7 cell line, including changes in the production of ERα mRNA, binding affinity, and ERα transcriptional activity [4]. In sexually naive adult female prairie voles, treatment with OT increased behavioral sensitivity to exogenous estrogen [15]. There are indications that neonatal manipulations of OT could have long lasting effects on the expression of behavioral and physiological responses regulated by estrogen [16].

In the present study, as a model we have used the prairie vole *(Microtus ochrogaster)*. This socially monogamous species exhibits behavioral characteristics that are similar to humans, including an active engagement in and reliance on the social environment, the formation of male–female pair bonds, display of biparental care, and a tendency to live in extended families [16–18]. In addition, recent evidence indicates remarkable parallels in mechanisms responsible for cardiovascular function in prairie voles and humans. For example, the neural control of the heart in prairie voles, as in humans, is strongly influenced by parasympathetic activity [19, 20].

In an attempt to define the possible role of OT receptor (OTR) and ERs during the early postnatal effects of OT on the heart, female and male prairie voles (*Microtus ochrogaster*) were treated with OT or OTA on the day of birth and the mRNAs expression for OTR, ERα and ERβ were measured on postnatal day 1 (D1), day 8 (D8) and day 21 (D21) utilizing reverse transcription-polymerase chain reaction (RT-PCR).

Results

Effect of neonatal OT or OTA administration on OT receptor, ERα and ERβ mRNAs expression in the heart postnatal day 1, day 8, and 21 prairie voles

Figure 1 shows a representative photographic negatives of agarose gel of the RT-PCR products for OTR mRNA (Fig. 1A) ERα mRNA (Fig. 1B) and ERβ mRNA (Fig. 1C).

OT receptor mRNA expression in the heart of postnatal day 1, day 8, and 21 prairie voles following neonatal OT or OTA administration

Neonatal treatment with OT significantly increased OTR mRNA expression in postnatal D1, but not D8, or D21 female (Fig. 2A) and male (Fig. 2B) animals compared with SAL-treated groups. OTR mRNA expression was significantly lower on postnatal D21 than D1 in both sexes. Although we found statistically significant differences in the level of OTR mRNA expression between ages of OTA-treated female and male animals, no effect of OTA treatment was detected when compared to SAL-treated groups (Fig. 2A, B).

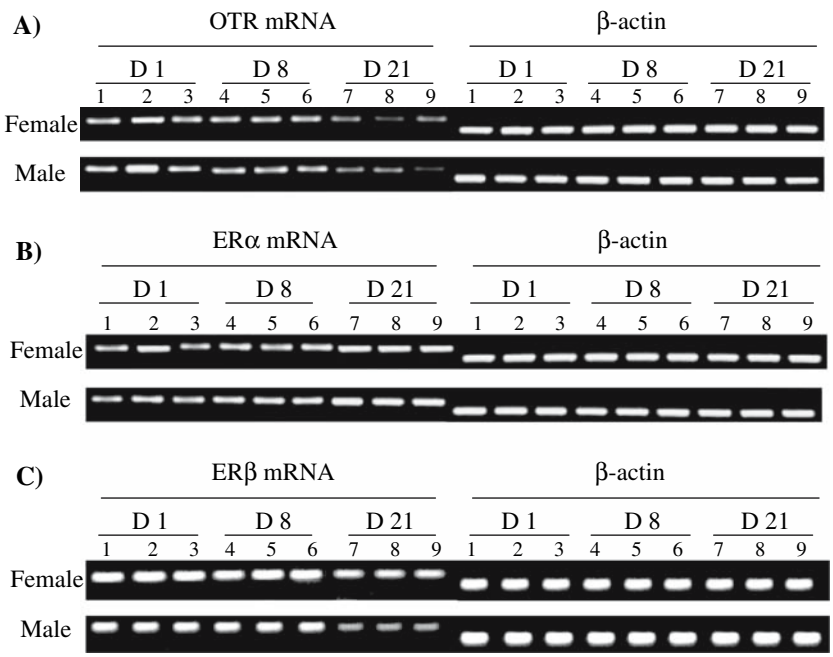

Fig. 1 A representative photographic negatives of agarose gel of the RT-PCR products for OTR mRNA (**A**), ERα mRNA (**B**) and ERβ mRNA (**C**) in the heart of postnatal day 1, 8, and 21 prairie voles following neonatal saline, OT, or OTA administration. RT-PCR was performed on RNA samples isolated from heart of female and male prairie voles after OT or OTA administration. β-actin was used as control to monitor the amount of mRNA in the samples. Lane 1: Saline, Lane 2: OT, Lane 3: OTA, Lane 4: Saline, Lane 5: OT, Lane 6: OTA, Lane 7: Saline, Lane 8: OT, Lane 9: OTA treatments

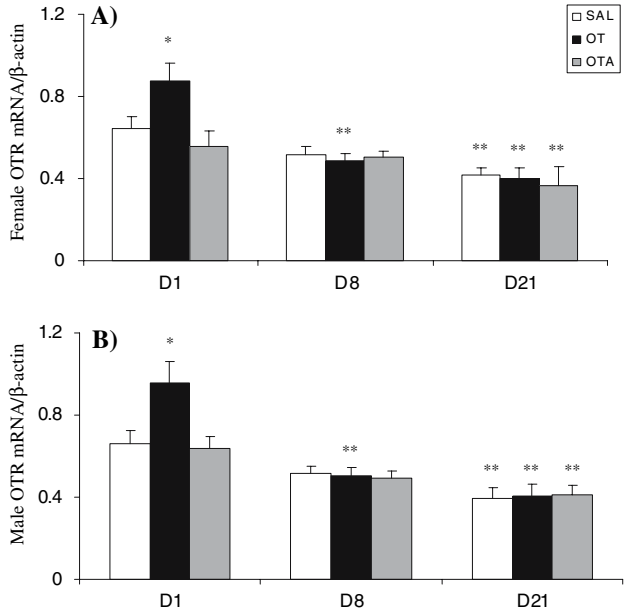

Fig. 2 Effect of neonatal treatment with OT or OTA on heart OTR mRNA expression on postnatal day 1, 8 or 21 in female (**A**) and male (**B**) prairie voles. Data represent means ± SEM ($n = 5$). * represents significant alteration at $P < 0.05$, when compared to SAL groups within the same age and sex; ** represents significant alteration at $P < 0.01$, when compared to D1 animals within the same treatment and sex

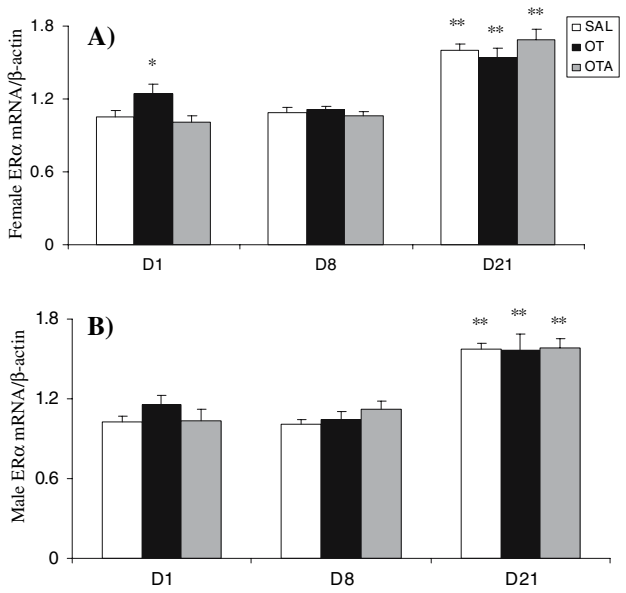

Fig. 3 Effect neonatal treatment with OT or OTA on cardiac ERα mRNA expression on postnatal day 1, 8 or 21 in female (**A**) and male (**B**) prairie voles. Data represent means ± SEM ($n = 5$). * represents significant alteration at $P < 0.05$, when compared to SAL group within the same age and sex; ** represents significant alteration at $P < 0.01$, when compared to D1 and D8 animals within the same treatment and sex

ERα mRNA expression in the heart of postnatal day 1, 8, and 21 prairie voles following neonatal OT or OTA administration

ERα mRNA expression was increased in the females (Fig. 3A), but not in males (Fig. 3B) on postnatal D1 following OT administration compared with SAL-treated pups. The ERα mRNA expression on postnatal D21 was significantly higher than those observed on D1 or D8. OTA administration showed no effect on heart ERα mRNA expression when compared to SAL-treated animals (Fig. 3A, B).

ERβ mRNA expression in the heart of postnatal day 1, 8, and 21 prairie voles following neonatal OT or OTA administration

While there was no effect of treatment on the expression of ERβ mRNA age-related changes were observed. These changes were opposite to those reported for ERα mRNA, with ERβ mRNA expression in postnatal D21 female (Fig. 4A) and male (Fig. 4B) animals being significantly lower than D1 and D8 animals in both sexes.

Discussion

In this study we examined the possible involvement of cardiac OTR and estrogen receptors (ERα and ERβ) mRNAs expression in the neonatal OT actions on the heart of postnatal D1, D8, and D21 female and male prairie voles. The results indicated that OTR and ERα mRNAs expression can be modulated by OT exposure in early postnatal life and this modulation is sexually dimorphic for ERα mRNA expression, with OT effects seen only in females. In addition to the potential effects of OT on cardiac development, we also found that there were significant changes in the expression of mRNAs for OTR, ERα and ERβ between the day of birth and the day of weaning (D21).

In the present study we observed that the basal level of heart OTR mRNA expression was significantly decreased by postnatal day 21, suggesting that cardiac OTR may be particularly functional in the fetal life, playing a lesser role as the animals develop. These findings are consistent with previous reports in rats showing that both OT and OTR are expressed in the fetal rat heart and are decreased to relatively low levels in adulthood [21]. Production of OT and the OTR in cardiac tissue during fetal life and early maturation may contribute to the development of the heart.

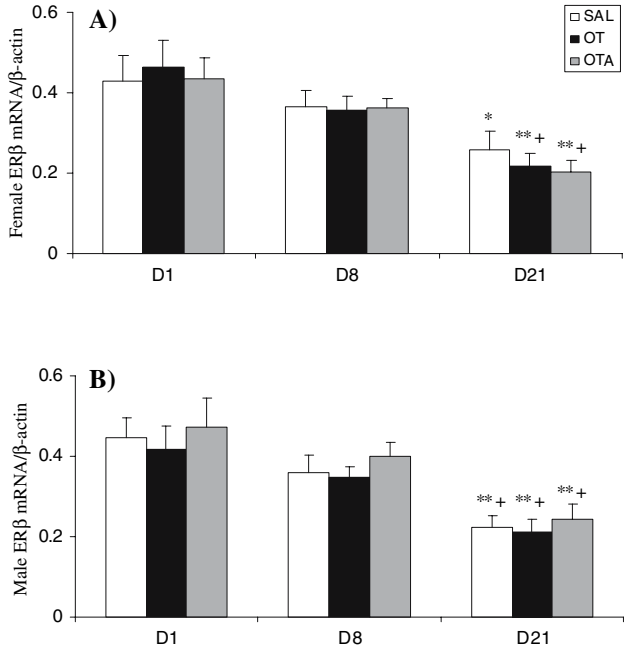

Fig. 4 Effect neonatal treatment with OT or OTA on heart ERβ mRNA expression on postnatal day 1, 8 or 21 in female (**A**) and male (**B**) prairie voles. Data represent means ± SEM (n = 5). * and ** represent significant alteration at $P < 0.05$ and $P < 0.01$, respectively, when compared to D1 animals within the same treatment and sex; + represents significant alteration at $P < 0.05$, when compared to D8 animals within the same treatment and sex

In addition, both OT synthesis [22] and the concentration of biologically active OT in the hypothalamus [21] are low on D1, but plasma OT levels in newborn rats exceed those found in adults [23]. It seems that cardiovascular synthesis of OT might supplement that of the hypothalamus. We found that OT administration caused a significant increase in heart OTR mRNA on D1, but not on D8 and D21, and OTA treatment elicited no changes in heart OTR mRNA expression, suggesting that OTR may be one possible factor that mediates the effects of OT on the cardiovascular system in early life. Further, support for a role of OT or the OTR comes from a study in P19 embryonic carcinoma cells, a model of mouse embryonic stem cells; these cells express OTRs, and OT stimulates the differentiation of the P19 cells into cardiomyocytes [24]. Further, an unpublished preliminary study from our laboratory showed that prenatal combination of OT and OTA treatment produced no statistically significant effects on cardiac OTRs in prairie voles. A previous study showed that perfusion of isolated rat hearts with OT induced significant bradycardia [6]. Oxytocin perfusion of isolated rat hearts results in a dose-dependent negative chronotropic effect while exerting a positive inotropic effect [10]. Further, it was reported that perfusion with OTA alone did not have any effect on heart rate and force of contraction. However, coadministration with OTA completely inhibited the effects of OT on beating rate and force, which suggested that these effects are mediated by OTRs [25]. It has been shown that stimulation of OTRs leads to elevation of intracellular Ca^{2+}. Increased Ca^{2+} stimulates cellular exocytosis [26] and also stimulates ANP secretion from the heart [27]. Due to the abundance of estrogen receptors in the rat heart [28] and the presence of estrogen receptors in myocytes secreting ANP [29], it was hypothesized that estrogen induces ANP synthesis and release from the heart through an ER-dependent mechanism. Since interactions between OT and estrogen have been reported in several systems, we also investigated the possible modulation of heart ERα and ERβ mRNAs expression in early postnatal life. Utilizing RT-PCR analysis, we found that ERs are developmentally regulated in the prairie vole heart. We observed that ERα expression rises from low levels on postnatal D1, whereas ERβ mRNA decreases by postnatal D21, suggesting a specific role for both receptors in heart maturation. We also found that OT administration on the first day of life caused a significant increase in heart ERα, but not in ERβmRNAs expression, and OTA treatment elicited no changes in heart ERs mRNAs expression, suggesting that ERα may be involved in the effects of OT on the cardiovascular system. Our current results are in agreement with a previous study in which high ERβ expression was observed in the newborn heart and on postnatal day 4 [30], when extensive hyperplasia of the rat heart occurs and the heart grows more rapidly than the body [31]. In adult rats, ERβ expression is low, in contrast to relatively high ERα mRNA levels. Age-dependent increases in ERα suggest that this receptor may play a role in heart maturation. Additional studies will be required to determine the mechanisms of differential modulation of ERs mRNAs between different ages, and the response of ERα mRNA between different sexes to neonatal OT manipulation. In summary, we have investigated the effects of early postnatal OT manipulation on heart OTR, and ERs mRNAs expression in neonatal or juvenile female and male prairie voles. We show that cardiac OTRs and ERα mRNAs expression can be rapidly modulated by OT exposure in early postnatal life and this modulation is sexually dimorphic for ERα mRNA expression.

Material and Methods

Animals and treatments

Animals used in this study were laboratory-reared F3 or F4 generation prairie voles that originated from wild stock trapped near Urbana, IL, USA. Within 24 h of birth, litters were sexed, toe-clipped for identification and culled to 6

pups. Litters were culled to ensure that litter size was uniform, so that feeding demands would be similar between litters. Only mixed-sex litters were used and single-sex litters were not created by culling. Female and male pups were weighed and randomly assigned to one of three treatment groups, with each treatment group being represented at least once per litter. The animals were maintained on a 12:12 h light/dark cycle and provided Purina rat chow and water *ad libitum*. They were housed in accordance with the USDA and NIH guidelines, and prior to conducting any research all procedures were approved by the University of Illinois at Chicago Animal Care and Use Committee. The procedures were designed to minimize both the number of animals used and potential pain and discomfort to the animals.

Treatment

On the day of birth (D1), pups (female and male) were temporarily removed from their parents and placed on cotton bedding and a lamp was used to maintain temperature. The pups were given a single intraperitoneal injection (50 µl volume) of isotonic saline (SAL), 3 µg OT/50 µl saline (OT), or 0.3 µg OT antagonist/50 µl saline (OTA) [[d(CH2)5, Tyr(Me)2, Orn8]-Vasotocin, Peninsula Laboratories, A Division of Bachem, Belmont, CA, USA], with an n of 5 per treatment. The dosage of OT was approximately 1 µg/g body weight and for OTA, 0.1 µg/g body weight. The doses of OT and OTA were chosen based on extensive literature indicating that during the neonatal period these doses can be affect a variety of physiological and behavioral responses in both rats and voles [32–35], as well as affect neuronal activation in neonates [36]. A lower dose of OTA (versus OT) was used here because the antagonist has a greater affinity for the OTR (greater than 10 times that of the natural ligand) [37]. Pups were returned as a group to the home cage within 10 min of removal. All pups were treated on the first day of life, and hearts were collected on postnatal D1 (2 h following treatment), D8 or D21. To collect the hearts, animals were deeply anesthetized, using a combination of ketamine and xylazine, and then decapitated. The heart was dissected on ice and frozen in liquid nitrogen and then stored at –80°C until RNA extraction. The tissues were collected during the lights on period between 11:00 and 13:00 h.

RNA extraction

Total RNAs were extracted from each frozen heart tissue following previously described methods [38] with slight modification. Briefly, frozen tissue was homogenized in 5–10 ml LiCl-urea (3 M LiCl, 6 M urea) per g tissue and incubated overnight at 4°C. Homogenate was then centrifuged at 8,000 rpm for 25 min. Supernatant was discarded and the walls of the tubes wiped with cotton swabs. Cold LiCl-urea was added to the tubes at half-volume and the process repeated. The resulting pellet was then dissolved in half volume 10 mM Tris pH 7.5, 1 mM EDTA, 0.5% SDS. An equal volume of phenol:choloroform:isoamyl alcohol [25:24:1] was added and vortexed. After centrifugation at 5000 rpm for 15 min, the upper aqueous layer was transferred to the new tubes, 100% EtOH was added to the each sample at 2x the volume plus 10 M of NH_4OAc at 1/10x the volume and mixed. Following precipitation and centrifugation at 5000 rpm for 25 min, the pellet was rinsed with 70% ETOH and re-suspended in 300 µl diethyl pyrocarbonate-treated (DEPC) water. The amount of RNA was estimated by spectrophotometry at 260 nm and the integrity of RNA was verified by agarose gel electrophoresis with ethidium bromide staining.

cDNA synthesis and polymerase chain reaction amplification (RT-PCR)

As we have described previously [39], cDNA synthesis and polymerase chain reaction (PCR) amplification for OTR, ERα & ERβ was performed using the ThermalCycler PCR. Briefly, total RNA (3 µg) was combined with 25 pmol oligo-d(T) primer, 0.5 µl RNase inhibitor, and the volume brought to 12 µl total with DEPC water. The samples were heated for 10 min at 70°C, placed on ice for 5 min, and then quickly centrifuged. To this mixture, was added 4 µl Superscript 5X first-strand buffer, 2 µl 0.1 M DTT, and 1 µl 10 mM dNTP mix (Roche (1 969 064). The samples were heated at 37°C for 2 min and 1 µl of Superscript RT added. This mixture was incubated at 42°C for 1 hr and then enzymes denatured by heating for 10 min at 95°C. The RT reaction was diluted 1:10 with DEPC water for PCR reaction. Exactly half of the first strand cDNA synthesis of each sample was used for PCR amplification, using primers designed to span at least one intron (to ensure cDNA and not contaminating genomic DNA is amplified). Polymerase chain reaction was performed in a total reaction volume of 100 µl containing oligonucleotide primers (0.2 µM), 1 × PCR buffer (10 mM Tris–HCL, 50 mM KCl, pH 8.3), 2 mM MgCl2, 0.2 m/m dNTPs, and 2.5 units of Taq DNA polymerase. The specific primers used were oxytocin receptor (OTR), estrogen-receptor alpha (ERα), estrogen receptor beta (ERβ) and β-actin, a 'house-keeping' gene used to determine the constitutive level of gene transcription and to control for variations in RNA recoveries. The β-actin primers were used simultaneously with each pair of primers (OTR, ERα and ERβ primers). The sequences for primers were as follow, for OTR (cDNA 370-bp), [sense 5′-TCTACATTGTACCG

GTCATCGTGC-3′, antisense 5′-AAGAGCATGGCAATG ATGAAGGCA-3′]; for ERα (cDNA 345-bp), [sense 5′-AATTCTGACAATCGACGCCAG-3′, antisense 5′-GTGC TTCAACATTCTCCCTCCTC-3′]; for ERβ (cDNA 210-bp), [sense 5′-GAAGCTGAACCACCCAATGT-3′, antisense 5′- CAGTCCCACCATTAGCACCT-3′] and for β-actin (cDNA 228-bp), [sense 5′-AGCCATGTACGTA GCCATCC-3′, antisense 5′-CTCTCAGCTGTGGTGGT-GAA-3′]. These primers were complementary of the mouse OTR (GenBank accession no. D86599), rat ERα (GenBank accession no. X61098), rat ERβ (GenBank accession no. X61098) and mouse β-actin (GenBank accession no. NM007393) genes. The reaction mixture was incubated in the thermal cycler for 33–35 cycles as follows: at 94°C for 60 s, at 56°C for 60 s and at 72°C for 60 s. As we have described previously [40], the cycles were terminated on the phase during which there was exponential generation of PCR product before reaching a plateau. When the cycles were completed, the tubes were maintained at 72°C for 5 min. Negation of contaminated genomic DNA was performed by analyzing the absence of templates in control samples without reverse transcriptase. Sequence analysis was performed to confirm the identity of the amplified cDNA.

Quantification of RT-PCR products

The RT-PCR method used in these assays is semi-quantitative because absolute values of mRNA levels could not be determined since internal controls for the specific mRNAs were not used. To validate this RT-PCR assay as a tool for the semiquantitative measurement of mRNA, dose-response curves were established for different amounts of total RNA extracted from the heart, and the samples were quantified in the curvilinear phase of PCR amplification. The expression levels were normalized against β-actin. No difference was observed in β-actin levels at any stage. 10 μl of the PCR product was analyzed on 0.8% agarose gel with ethidium bromide. RT-PCR amplification of OTR, ERα and ERβ in the heart revealed PCR products of the expected base pair for each primer. The quantification of RT-PCR products was performed by densitometric analysis of photographic negatives of agarose gels using NIH Image and then the ratios of OTR, ERα or ERβ/β-actin were calculated.

Statistical analysis

Data are presented as means ± SEM. Statistical comparisons were made using two-way ANOVA. If significance was observed, posthoc pair wise comparisons were made using Fisher's protected least squares difference test. A level of $P < 0.05$ was accepted as statistically significant. Statistical values are shown in Figure legends.

Acknowledgments We thank Dr. Angela J. Grippo, Brain-Body Center, College of Medicine, University of Illinois at Chicago for her valuable comments on the manuscript. This study was supported by National Institute of Child Health and Human Development HD38490 (CSC) and National Institute of Mental Health MH073022 (CSC).

References

1. V. Du Vigneaud, C. Ressler, S. Trippett, J. Biol. Chem. **205**, 949 (1953)
2. D.L. Lefebvre, A. Giaid, H. Bennett, R. Lariviere, H.H. Zingg, Science **256**, 1553 (1992)
3. M. Jankowski, F. Hajjar, S. Al-Kawas, S. Mukaddam-Daher, G. Hoffman, S.M. McCann, J. Gutkowska, Proc. Natl. Acad. Sci. USA **95**, 14558 (1998)
4. F. Moos, D.A. Poulain, F. Rodriguez, Y. Guerne, J.D. Vincent, P. Richard, Exp. Brain Res. **76**, 593 (1989)
5. M. McCarthy, L.M. Kow, D. Pfaff, Ann. NY Acad. Sci. **652**, 70 (1992)
6. J. Gutkowska, M. Jankowski, C. Lambert, S. Mukaddam-Daher, H.H. Zingg, S.M. McCann, Proc. Natl. Acad. Sci. USA **94**, 11704 (1997)
7. M. Thibonnier, D.M. Conarty, J.A. Preston, C.L. Plesnicher, R.A. Dweik, S.C. Erzurum, Endocrinology **140**, 1301 (1999)
8. K.P. Conrad, M. Gellai, W.G. North, H. Valtin, Ann. NY Acad. Sci. **689**, 346 (1993)
9. M.A. Haanwinckel, L.K. Elias, A.L. Favaretto, J. Gutkowska, S.M. McCann, J. Antunes-Rodrigues, Proc. Natl. Acad. Sci. USA **92**, 7902 (1995)
10. C.C. Coulson, J.M. Thorp Jr., D.C. Mayer, R.C. Cefalo, Am. J. Obstet. Gynecol. **177**, 91 (1997)
11. J.A. Gustafsson, J. Endocrinol. **163**, 379 (1999)
12. Y.K. Hodges, L. Tung, X.D. Yan, J.D. Graham, K.B. Horwitz, L.D. Horwitz, Circulation **101**, 1792 (2000)
13. P. Cassoni, A. Sapino, N. Fortunati, L. Munaron, B. Chini, G. Bussolati, Int. J. Oncol. **72**, 340 (1997)
14. P. Cassoni, M.G. Catalano, A. Sapino, T. Marrocco, A. Fazzari, G. Bussolati, N. Fortunati, Int. J. Oncol. **21**, 375 (2002)
15. B.S. Cushing, C.S. Carter, J. Neuroendocrinol. **11**, 765 (1999)
16. C.S. Carter, Physiol Behav. **79**, 383 (2003)
17. C.S. Carter, A.C. DeVries, L.L. Getz, Neurosci. Biobehav. Rev. **19**, 303 (1995)
18. E.B. Keverne, J.P. Curley, Curr. Opin. Neurobiol. **14**, 777 (2004)
19. A.J. Grippo, D.G. Lamb, C.S. Carter, S.W. Porges, Physiol. Behav. **90**, 386 (2007)
20. A.J. Grippo, B.S. Cushing, C.S. Carter, Psychosom. Med. **69**, 149 (2007)
21. M. Jankowski, B. Danalache, D. Wang, P. Bhat, F. Hajjar, M. Marcinkiewicz, J. Paquin, S.M. McCann, J. Gutkowska,, Proc. Natl. Acad. Sci. USA **101**, 13074 (2004)
22. G. Almazan, D.L. Lefebvre, H.H. Zingg, Dev. Brain Res. **45**, 69 (1989)
23. R.D. Hartman, L.M. Rosella-Dampman, S.E. Emmert, J.Y. Summy-Long, Endocrinology **119**, 1 (1986)
24. J. Paquin, B.A. Danalache, M. Jankowski, S.A. McCann, J. Gutkowska, Proc. NatL. Acad. Sci. USA **99**, 9550 (2002)
25. S. Mukaddam-Daher, Y.L. Yin, J. Roy, J. Gutkowska, R. Cardinal, Hypertension **38**, 292 (2001)

26. D.E. Knight, H. von Grafenstein, C.M. Athayde, Trends Neurosci. **12**, 451 (1989)
27. H. Ruskoaho, M. Toth, R.E. Lang, Biochem. Biophys. Res. Commun. **133**, 581 (1985)
28. W.E. Stumpf, M. Sar, G. Aumuller, Science **196**, 319 (1977)
29. H. Back, W.G. Forssmann, W.E. Stumpf, Cell Tissue Res. **255**, 673 (1989)
30. M. Jankowski, G. Rachelska, D. Wang, S.A. McCann, J. Gutkowska, Proc. Natl. Acad. Sci. USA **98**, 11765 (2001)
31. F.J. Clubb, S.P. Bishop, Lab. Invest. **50**, 571 (1984)
32. K. Uvnäs-Moberg, P. Alster, M. Petersson, A. Sohlstrom, E. Bjorkstrand, Pediatr Res. **43**, 344 (1998)
33. K.M. Kramer, B.S. Cushing, C.S. Carter, Physiol. Behav. **79**, 775 (2003)
34. B.S. Cushing, K. Levine, N.L. Cushing, Horm. Behav. **47**, 22 (2004)
35. Y. Yamamoto, B.S. Cushing, K.M. Kramer, P.D. Epperson, G.E. Hoffman, C.S. Carter, Neuroscience **125**, 947 (2004)
36. B.S. Cushing, Y. Yamamoto, G.E. Hoffman, C.S. Carter, Dev. Brain Res. **143**, 129 (2003)
37. C. Barberis, E. Tribollet, Crit. Rev. Neurobiol. **10**, 119 (1996)
38. C. Auffray, F. Rougeon, Eur. J. Biochem. **107**, 303 (1980)
39. H. Pournajafi Nazarloo, T. Takao, W. Nanamiya, K. Asaba, E.B. De Souza, K. Hashimoto, Brain Res. **902**, 119 (2001)
40. H. Pournajafi-Nazarloo, M. Carr, E. Papademeteriou, J. Schmidt, B. Cushing, Neuropeptides **41**, 39 (2007)