Neuroscience Letters 454 (2009) 118–123

Contents lists available at ScienceDirect

Neuroscience Letters

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

Age-related changes in dorsal root ganglia, circulating and vascular calcitonin gene-related peptide (CGRP) concentrations in female rats: Effect of female sex steroid hormones

Pandu R.R. Gangula[a,*], Madhu Chauhan[b], Luckey Reed[b], Chandra Yallampalli[b]

[a] Department of Obstetrics and Gynecology, Meharry Medical College, 1005 Dr. D.B. Todd Jr. Boulevard, Nashville, TN 37208, United States
[b] Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, United States

ARTICLE INFO

Article history:
Received 22 January 2009
Received in revised form 20 February 2009
Accepted 28 February 2009

Keywords:
Dorsal root ganglia
Young adult
Aging
CGRP
Estrogen
Progesterone
Circulation
Vasculature

ABSTRACT

The aim of the present study is to investigate whether immunoreactive (I) calcitonin gene-related peptide (CGRP) content is decreased in plasma and mesenteric arteries (resistance arteries) in middle-aged rats and if so, whether sex steroid hormones enhance I-CGRP in middle-aged female rats. We also examined whether vascular CGRP receptor components, calcitonin receptor like receptor (CRLR) and receptor activity modifying protein 1 ($RAMP_1$) are elevated by sex steroid hormones treatment in middle-aged female rats. Young adult (3 months old) and middle-aged (10–12 months old) ovariectomized rats were treated subcutaneously with estradiol-17β (E_2; 2 mg), progesterone (P_4; 5 mg), $E_2 + P_4$ (2 mg + 20 mg) or placebo (control). Radioimmunoassay and Western blot analysis were performed to measure I-CGRP content and CGRP receptor components in dorsal root ganglia (DRG), in resistance arteries and in plasma. Immunofluorescent staining methods were employed to determine cellular localization of CRLR, $RAMP_1$ in resistance arteries. Our data demonstrated that I-CGRP content was significantly ($p < 0.05$) lower in the plasma and resistance arteries of middle-aged female rats compared to young controls. Both $RAMP_1$ and CRLR were concentrated in vascular endothelium and the underlying smooth muscle cells. $RAMP_1$ but not CRLR appeared to be decreased in middle-aged rat vasculature. Chronic perfusion of sex steroid hormones to ovariectomized rats: (1) significantly ($p < 0.05$) elevated I-CGRP in the DRG and in the plasma, and (2) significantly elevated $RAMP_1$ ($p < 0.05$) but did not alter CRLR in resistance arteries. These data suggest that female sex steroid treatment enhances I-CGRP and its receptors, and thus regulate the blood pressure in aged female rats.

Published by Elsevier Ireland Ltd.

Aging is one of the determinants of blood pressure (BP) variability and possibly contributes to the pathogenesis of essential hypertension and cardiovascular disease. The observation that BP is low in pre-menopausal women compared to age matched men and older women suggested that female sex steroid hormone therapy (HT) might influence hypertension in post-menopausal women [10]. Although, reports from Heart and Estrogen–Progestin Replacement Study (HERS), HERS2, and Women's Health Initiative (WHI) studies do not support beneficial vascular effects of HT, particularly in elderly hypertensive women, several studies have suggested the beneficial effects of HT on reducing cardiovascular diseases in this population [9,10,37,39].

Hypertension is a multi-factorial disorder. A number of mechanisms have been proposed for age-related increases in BP. Diminished baroreflex compensation is responsible for the failure to respond appropriately to rapid change in the BP, whereas increased vascular stiffness and diminished glomerular filtration may be responsible for the gradual increase in BP. Previous studies in spontaneous hypertensive rats (SHR) [2] and Fischer 144 aging rats demonstrated that endothelium-dependent relaxation is reduced compared to young adult group [2]. These studies further suggested that nitric oxide (NO) synthesis/release by endothelial cells and thereby the activity of the nitric oxide–cyclic gunaosine monophosphate (NO–cGMP) pathway in smooth muscle is reduced in aging rats. In addition, recent studies demonstrated that estrogen replacement in aged bilaterally ovariectomized (OVX) rat restored impaired vascular relaxation and NO signaling [44]. The above studies suggest that age-related endothelial dysfunction may contribute to the increase in arterial pressure.

The BP lowering effects of estrogen therapy (ET) has been reported in post-menopausal women as well as in animal models [10,39,54]. In addition, reports indicate [10,39] that pre-menopausal women have a high cardiac output and lower total peripheral resistance compared to men of same age, and potent

* Corresponding author. Tel.: +1 615 327 6511.
E-mail address: pgangula@mmc.edu (P.R.R. Gangula).

0304-3940/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.neulet.2009.02.068

vasodilator effects of estrogens (E_2) have been shown in several *in vivo* [11,19,40] and *in vitro* [11,12,36] studies. Similarly, potent vascular protective effects of progesterone (P_4) are also documented in both rats [46] and human [23,51]. Taken together, these studies suggest that decreases in hormone levels may be responsible for BP increase in aged population. In addition to NO and prostaglandins, it has been proposed that calcitonin gene-related peptide (CGRP) is also one of the mechanisms regulating BP lowering effects of sex steroid hormones [13,17,20].

CGRP is a potent vasodilator neuropeptide and is produced by the tissue-specific alternative splicing of the primary transcript of the calcitonin/CGRP gene [52]. This peptide is distributed throughout the central and peripheral nervous systems and is located in areas that are involved in cardiovascular functions [3,31,53]. CGRP is released from the never terminals, binds to its receptors on the vasculature [48,52], and reduces the BP via peripheral vasodilation [21,34]. Studies from our laboratory have been shown that both circulatory CGRP levels [14] and receptors in vasculature [55] are elevated by E_2 and P_4 treatment in young adult female rats. The expression of CGRP in dorsal root ganglia (DRG), the prominent site of CGRP synthesis is regulated by female sex steroid hormones [15]. Vasodilatory effects of CGRP are significantly improved by sex steroid hormones in both young adult [18] and aged female [17] rats. Furthermore, we have reported that P_4 modulated the vasodilator effects of CGRP in the presence of N^G-nitro-L-arginine methyl ester [L-NAME, the inhibitor of nitric oxide], indicating a NO-independent pathway [16]. Collectively, theses studies suggest that vasodilator effects of CGRP are modulated by sex steroid hormones in both young adult and aged rats. Lu et al. [28] have demonstrated that the circulatory levels of both E_2 and P_4 are reduced in aged female rats. Moreover, previous reports postulated that CGRP concentrations in the circulation were elevated by HT in post-menopausal women [43,47]. However, it is not clear whether both dorsal root ganglia and circulatory CGRP content and its receptors are altered in aging vasculature and if so, whether HT restores this effect. We hypothesize that decreases in the sex steroid hormone levels in the circulation might down-regulate the synthesis and/or release of CGRP, and its receptors on the vasculature and develop hypertension in older women. The objectives of the present study are to investigate whether: (1) the levels of CGRP in the circulation, in resistance blood vessels, and CGRP receptors in mesenteric arteries are altered with aging; (2) steroid hormone treatments can modulate CGRP system in aged rat model.

Young adult (3 months old) non-pregnant or middle-aged (10–12 months old) rats were purchased from Harlan Sprague Dawley (Houston, TX, USA) and housed in a climatic controlled room with a 12L:12D schedule. Animals received an ad libitum supply of rat chow and water. All procedures were approved by the Animal Care and Use Committee of the University of Texas Medical Branch, Galveston, TX, USA.

Groups of 4–6 young adult or middle-aged female rats were either left intact (ovary-intact) or bilaterally ovariectomized while under ketamine (45 mg/kg b.w.; Fort Dodge Laboratory, Fort Dodge, IA) and xylazine (5 mg/kg b.w.; Burns Veterinary Supplies, New York, NY) anesthesia. Seven days after OVX, animals were implanted sc (per kg body weight) with 21-day release of E_2 (2 mg), P_4 (20 mg), $E_2 + P_4$ (2 mg + 20 mg), or placebo (control). These doses were shown to achieve physiological concentrations in the circulation in both young adult and aged rats [4]. After 7 days of treatment (10–11 a.m.) blood was collected into heparin zed tubes from the vena cava immediately following exposure to CO_2 in an inhalation chamber. Blood was then centrifuged at 1600 × *g* for 15 min at 4 °C, and plasma was collected and stored at −70 °C until used. Dorsal root ganglia and mesenteric arteries from both young adult and middle-aged rats were removed immediately, quickly frozen in liquid nitrogen and stored at −70 °C until used. On the experimental day the young adults were in diestrus, and middle-aged rats were at persistent diestrus stage. The stage of estrus cycle was assessed by examining cells in a vaginal flush using light microscopy.

Table 1
Changes of plasma estradiol-17β (E_2) and progesterone (P_4) concentrations in young adult rats (3 months old) during diestrus stage of estrus cycle or persistent diestrus middle-aged (10–12 months old) rats. Sex steroid hormone levels in plasma were measured by radioimmunoassay. Values are mean ± SEM for 4–6 animals in each group.

Group	Sex steroid hormones in plasma	
	Estradiol-17β (pg/mL/kg b.w.)	Progesterone (ng/mL/kg b.w.)
Young adult female rat (3 months old)	231 ± 29	95 ± 12
Middle-aged female rat (10–12 months old)	71 ± 4*	36 ± 4*

* $p < 0.05$ compared to young adult group.

Plasma was processed for the measurement of E_2 (pg/mL/kg b.w.) and P_4 (ng/mL/kg b.w.) using specific ^{125}I label for E_2 or P_4, respectively, by radioimmunoassay (RIA) method as per instructions provided by supplier (Diagnostic Systems Laboratories, Webster, TX, USA).

The extraction of I-CGRP from plasma, dorsal root ganglia and mesenteric arteries were followed as published earlier [14,43,47]. Briefly, acidified plasma supernatants were pooled and passed through Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA, USA) primed with 100% acetonitrile. The elutes were vacuum-dried and stored at −70 °C for measurement of I-CGRP by RIA using CGRP RIA Kit (Phoenix Pharmaceuticals Inc., Belmont, CA, USA). The dried elute was reconstituted in a small amount (15–30 μL) of 0.1% TFA over a period of 30 min at 4 °C. The CGRP content in plasma was expressed as pmol/L.

Dorsal root ganglia and mesenteric arteries (pg/mg) I-CGRP content was calculated from total protein in the supernatant, which was measured by BCA Kit (Pierce, Rockford, IL, USA). Equal amounts of protein were used in duplicate to determine I-CGRP levels.

I-CGRP was measured by radioimmunoassay utilizing a rabbit antiserum raised against synthetic human α-CGRP conjugated to bovine albumin. The sensitivity was 32 pg/tube and the intra-assay and inter-assay variations were 5% and 10%, respectively, and the cross-reactivity for the antibody used was 100% and 35.5% for rat α-CGRP and human α-CGRP, respectively.

Mesenteric arteries were thawed on ice before homogenization. Experimental protocols for calcitonin receptor like receptor (CRLR) and receptor activity modifying protein 1 ($RAMP_1$) protein expressions were followed as reported previously [4]. Densitometric analyses were performed in the linear range using a Fluorchem Analysis System (Alpha Innotech, San Leandro, CA, USA).

Table 2
Changes in plasma and mesenteric artery immunoreactive calcitonin gene-related peptide (I-CGRP) concentrations in young adult rats (3 months old) during diestrus stage of estrus cycle or persistent diestrus middle-aged (10–12 months old) rats. I-CGRP in plasma and mesenteric arteries were measured by radioimmunoassay. Values are mean ± SEM for 4–6 animals in each group.

Group	Immunoreactive CGRP (I-CGRP)	
	Plasma (pmol/L)	Mesenteric artery (pg/mg protein)
Young adult female rat (3 months old)	14.0 ± 0.2	390 ± 23
Middle-aged female rat (10–12 months old)	10 ± 0.1*	202 ± 12*

* $p < 0.05$ compared to young adult group.

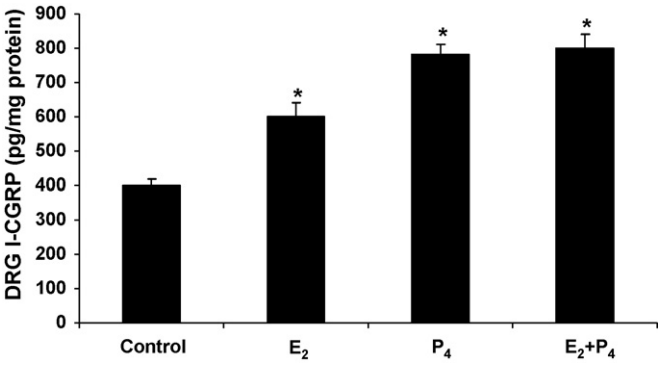

Fig. 1. Effects of estradiol-17β (E_2), progesterone (P_4) either alone or in combination on the dorsal root ganglia (DRG) immunoreactive calcitonin gene-related peptide (I-CGRP) in middle-aged (10–12 months old) female rats. After 1 week of ovariectomy, animals were implanted subcutaneously (per kilogram body weight) with 21-day release of either E_2 (2 mg), P_4 (20 mg), $E_2 + P_4$ (2 mg + 20 mg), or placebo (control) pellets and blood was collected after 7 days of treatment for the measurement of I-CGRP levels in the DRG by radioimmunoassay method. Data were mean ± SEM for 4–6 animals in each group. *$p < 0.05$ compared with control group.

Young adult or middle-aged female rat mesenteric arteries were fixed in BOUIN's fixative [8]. After a routine tissue processing procedure of dehydration in ascending grades of ethanol, cleaning in xylene, and infiltration with paraffin, the tissue were embedded in paraffin. Sections (5 μM thick) were rinsed with 3% normal goat serum and Triton x-100 for 30 min at room temperature and then incubated with avidin–biotin blocking buffer to reduce non-specific staining. The primary polyclonal antibody for CRLR and $RAMP_1$ in 1% normal goat serum was applied to the slides and incubated overnight in the cold room (4 °C). After being washed with PBS, the slides were incubated with the fluorescence-conjugated secondary antibody Alexa Fluor 594 (Molecular Probes, Eugene, OR, USA) at room temperature for 4 h. The slides were then rinsed with PBS, mounted using 4′,6-diamidino-2-phenylindole (Vector Laboratories; Burlingame, CA, USA), covered with coverships, and viewed under an Olympus microscope (Olympus Optical, Tokyo, Japan).

Results are expressed as the mean ± SEM. Data were analyzed for statistical differences with the Student's *t*-test or one-way ANOVA followed by the Bonferroni *t*-test to verify differences between individual groups. Differences were considered to be significant if $p < 0.05$ ($n = 4–6$).

Results show that plasma levels of E_2 (pg/mL/kg b.w.) are significantly ($p < 0.05$) decreased in middle-aged female rats (71 ± 4.0) compared to the young adult group (231 ± 29) (Table 1). Similarly, the plasma levels of P_4 (ng/mL/kg b.w.) in young adult circulation were greater (95 ± 12) compared to middle-aged group (36 ± 4) (Table 1). The levels of I-CGRP in middle-aged rat circulation (pmol/L) were significantly ($p < 0.05$) reduced (10.0 ± 0.1) compared to young adult group (14.0 ± 0.2) (Table 2). Similarly, significant decreases in mesenteric I-CGRP content (pg/mg protein) were observed in middle-aged rats (202 ± 12) compared to young adult group (390 ± 23).

We observed that DRG I-CGRP levels (pg/mg tissue) were significantly ($p < 0.05$) elevated in E_2 (600 ± 40), P_4 (780 ± 30) and $E_2 + P_4$ (800 ± 40) treated young adult OVX rats compared to placebo (control) treated (400 + 20) group (Fig. 1).

As shown in Fig. 2A, plasma I-CGRP levels (pmol/L) were significantly ($p < 0.05$) elevated in E_2 (16.1 ± 1.6), P_4 (15.4 ± 0.9) and $E_2 + P_4$

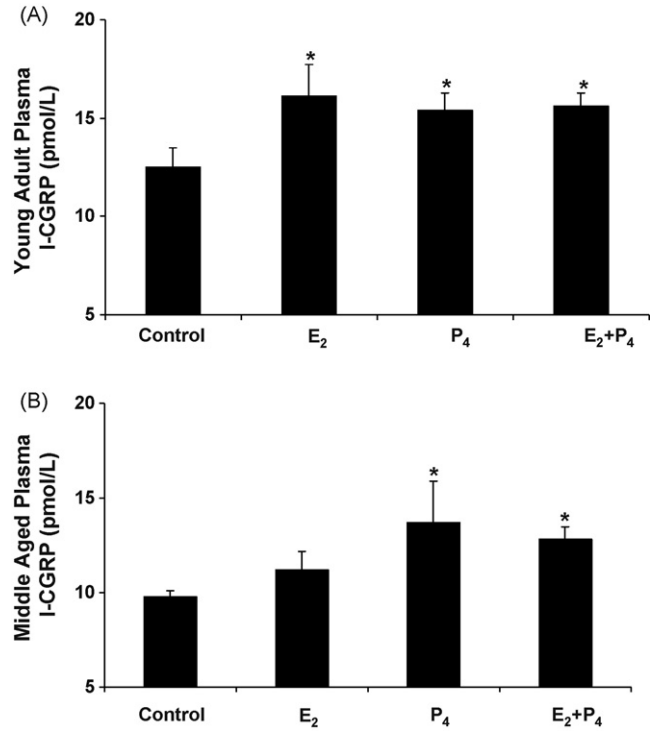

Fig. 2. Effects of estradiol-17β (E_2), progesterone (P_4) either alone or in combination on the plasma immunoreactive calcitonin gene-related peptide (I-CGRP) concentrations in young adult (A, 3 months old) or in middle-aged (B, 10–12 months old) female rats. After 1 week of ovariectomy, animals were implanted subcutaneously (per kilogram body weight) with 21-day release of either E_2 (2 mg), P_4 (20 mg), $E_2 + P_4$ (2 mg + 20 mg), or placebo (control) pellets and blood was collected after 7 days of treatment for the measurement of I-CGRP levels in the circulation by radioimmunoassay method. Data were mean ± SEM for 4–6 animals in each group. *$p < 0.05$ compared with control group.

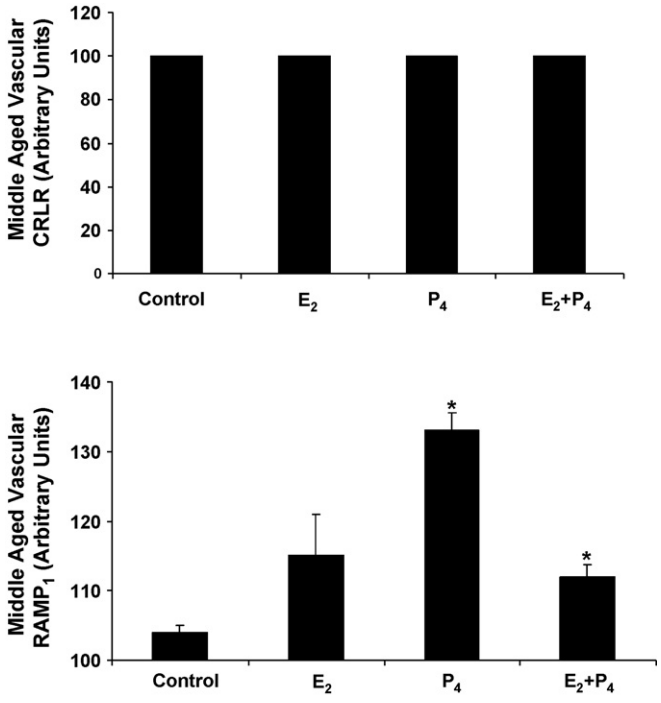

Fig. 3. Effects of estradiol-17β (E_2), progesterone (P_4) either alone or in combination on calcitonin gene-related peptide receptor component protein expression in middle-aged (10–12 months old) ovariectomized (OVX) rat mesenteric arteries. The OVX animals were implanted subcutaneously (per kilogram body weight) with 21-day release of either E_2 (2 mg), P_4 (20 mg), $E_2 + P_4$ (2 mg + 20 mg), or placebo (control) pellets and mesenteric arteries were collected after 7 days for Western blot analysis. Densitometric analysis for CRLR (58 kDa) (A) and $RAMP_1$ (14 kDa); (B) are presented in the bar graph. The bars represent mean ± SEM ($n = 4–6$). Groups with asterisk at the top of the bars differ significantly ($p < 0.05$) from placebo (control).

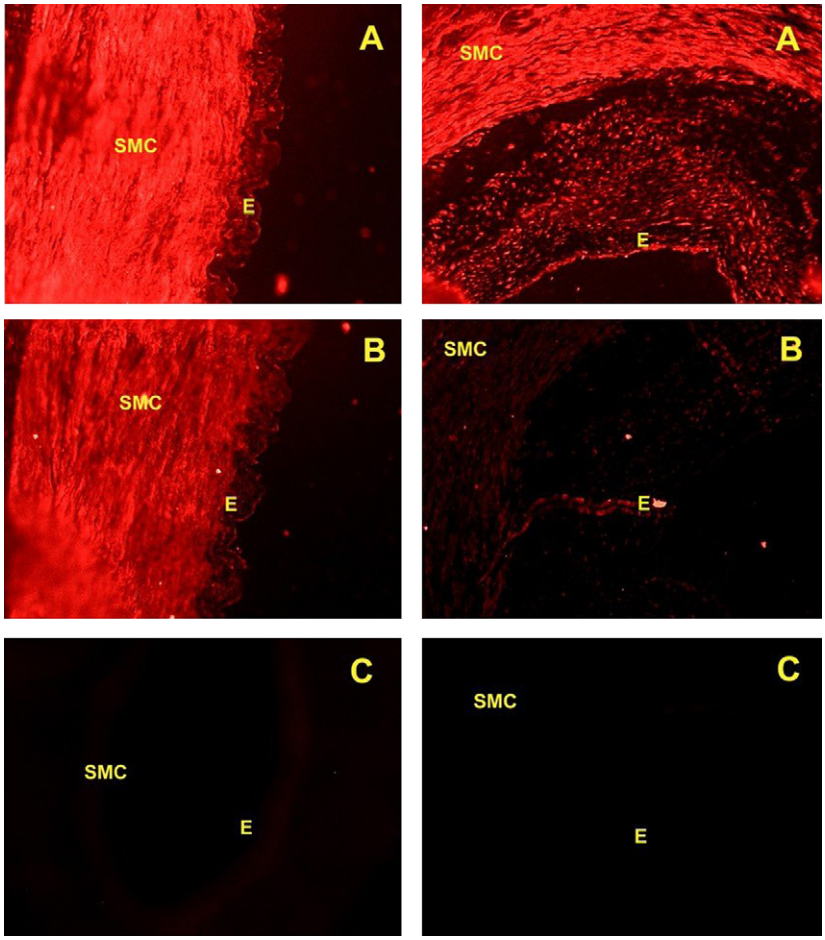

Fig. 4. Immunofluorescent localization of CGRP receptor components; CRLR [A1 (left), A2 (right)] and $RAMP_1$ [B1 (left), B2 (right)] in female rat mesenteric arteries. Sections (×100) from young adult during diestrus (left panel; 2 and 3 months old) stage and middle-aged during persistent diestrus (right panel; 10–12 months old) stage were used. E, endothelium; SMC, smooth muscle cells. None of the sections examined showed positive immunostaining when primary antibody was omitted (−antibody) [C1 (left), C2 (right)].

(15.6 ± 0.7) treated young adult OVX rats compared to placebo (control) treated (12.5 + 1.0) group. Similar trends were also observed in circulatory I-CGRP levels in the aged-OVX female rats upon E_2 (11.2 ± 1.0), P_4 (13.7 ± 2.2) or $E_2 + P_4$ (12.8 ± 0.7) treatments compared to the control group (9.8 ± 0.3) (Fig. 2B).

Densitometric analysis revealed that the receptor protein expression for CRLR in aged rat mesenteric arteries did not change in the presence of these hormones (Fig. 3A). However, the protein expression for $RAMP_1$ was substantially elevated by treatment with E_2, P_4 either alone or in combination compared to control group (Fig. 3B).

Using immunofluorescent staining, we found that both CRLR (Panel A) and $RAMP_1$ (Panel B) were present in resistance arteries, with staining localized to both endothelium and underlying smooth muscle cells. These results further show that staining for $RAMP_1$ (Panel B, right), but not CRLR (Panel A, right) appeared to be decreased in middle-aged female rats compared to young adults (CRLR, left panel A; $RAMP_1$, left panel B) (Fig. 4).

The present study confirmed the previous reports that circulatory levels of sex steroid hormones decreased in both post-menopausal women [43,47] as well as in aged rats [28] compared to pre-menopausal women and young adult rats, respectively (Table 1). We hypothesized that decreases in circulatory hormone concentrations might reduce the CGRP levels as well, therefore increase the BP in aged population. In this study, we found that both plasma and resistance artery I-CGRP levels are significantly ($p < 0.05$) decreased in ovary-intact middle-aged rats compared to young adult animals (Table 2). Previous studies demonstrated that the levels of I-CGRP in mesenteric (resistance) and femoral arteries are lower in older male rats than in young adults [25]. CGRP immunoreactivity was decreased at the lumbar 4 to 5 levels of the dorsal horn of the spinal cord in aged male rats [1]. However, none of these studies, except our current findings, addressed whether decreases in circulatory levels of E_2 and P_4 influence the CGRP levels in middle-aged female rats. These studies together with our current findings suggest that CGRP synthesis in DRG in middle-aged rats may be decreased; therefore the levels of this peptide were reduced in both mesenteric vasculature and in the circulation.

It has been well-documented that HT is known to decrease BP in post-menopausal women [10,39], SHR [45,49] and in young adult OVX rats [54]. The pathways by which hormones interact with the vascular physiology are not completely understood. Our recent studies demonstrated the BP lowering effects of CGRP are amplified in the presence of hormones in both OVX young adult [17] and aged female rats [18]. We postulated that sex steroid hormone elevate the synthesis and/or release of treatment of I-CGRP into the circulation and therefore decreased the BP. In the current study, we observed that sex steroid hormone pellets (this concentration was shown to achieve physiological levels in the rat circulation)

[4], significantly ($p < 0.05$) elevated I-CGRP levels in middle-aged female rat DRG (Fig. 1). These studies provide additional evidence that steroid hormones increase CGRP synthesis both in young adult and in the aged population.

In the present study, we observed that treatment with E_2 ($p < 0.07$), P_4, and $E_2 + P_4$ treatments significantly ($p < 0.05$) elevated plasma levels of I-CGRP in middle-aged (10–12 months old) female (Fig. 2B) rats similar to that of young adult group (Fig. 2A). In a preliminary study, we have noticed that E_2 treatment significantly ($p < 0.01$) elevated plasma I-CGRP content in older (16–18 months old) female rats (40 ± 2) compared to either intact or OVX (10 ± 0.08) control groups. Additional studies are warranted to evaluate dose- and age-dependent effects of sex steroids on I-CGRP content in DRG, mesenteric vasculature and in the circulation. Previous studies demonstrated that HT elevates I-CGRP levels in circulation of post-menopausal women compared to pre-menopausal state [43,47]. Collectively, these data suggest that HT increases both DRG and circulatory I-CGRP levels independent of age and thus maintain the vascular tone.

CRLR forms a high-affinity receptor for CGRP when co-expressed with $RAMP_1$ [32]. In turn, $RAMP_1$ acts as a chaperone protein, is required for correct routing of CRLR to the cell surface, and contributes to the pharmacological specificity of CRLR. Because the vasodilator sensitivity to CGRP and I-CGRP levels in circulation are elevated by female sex steroid hormone treatment, we next examined whether the protein expression of CRLR and $RAMP_1$ are modulated by these hormones. We found that $RAMP_1$ (Fig. 3B) but not CRLR (Fig. 3A) protein in mesenteric vessels were elevated by P_4 either alone or in combination with E_2 in middle-aged female rats. However, the expression of $RAMP_1$ was substantially ($p < 0.07$) but not significantly elevated by E_2 treatment in middle-aged female rats. Furthermore, data obtained from immunofluorescent staining shows that $RAMP_1$ but not CRLR expression appeared to be decreased in middle-aged rat vasculature (Fig. 4). Additional studies are required to demonstrate the age- and dose-dependent effects of sex steroid hormones on vascular CGRP receptors expression.

Coronary heart disease (CHD) is the leading cause of death in women in the United States. The risk for coronary heart disease is much higher in African–American (AA) women than in Caucasians [5–7] due to, at least in part, to lower levels of circulating sex hormones in AA women [24,29,33]. Previous studies demonstrated only the changes in circulatory I-CGRP with or without hormone therapy in post-menopausal women [16,28]. However, these studies have not discussed whether racial/ethnic differences are exist with I-CGRP, CGRP receptor components, vasodilator responses to CGRP, in parallel with circulatory estrogens and if so whether this system is altered with hormone therapy. Our long-term goal is to test the hypothesis that the CGRP system is the mechanism involved in vasoprotection due to circulating sex hormones, and that appropriate HT, may lead to successful protection against CHD in post-menopausal women. Although, reports from Heart and Estrogen–Progestin Replacement Study, HERS2, and Women's Health Initiative studies do not support beneficial vascular effects of HT, these studies [37,39] together with recent emerging and national register study [9,22,26,27,30,35,41,42,50] could not rule out the possibility that depend upon the CV condition and/or age, other formulations of oral estrogens, phyoestrogens and progesterone's and/or transdermal estradiol with progesterone at physiological doses may provide a different risk–benefit profile. The above studies also suggest that early initiation of therapy would be beneficial and not harmful. Therefore, the future studies will be focused on (1) to investigate dose, route, type of hormone regimen and (2) duration of ET and/or HT that lower the risk for CHD by stimulating endogenous vasoactive hormones such as CGRP in post-menopausal women.

Acknowledgements

We thank Cheryl Welch for typing the final manuscript. This work is supported by NIH-NIA – R03AG018999-01 awarded to Pandu R.R. Gangula. We also would like to thank Dr. Veera Rajaratnam, Director of Scientific Publications and Grant Support Core at the Center for Women's Health Research, Meharry Medical College, for the excellent editing and proof reading of the revised manuscript.

References

[1] E. Bergman, H. Johnson, X. Zhang, T. Hokfelt, B. Ulfhake, Neuropeptides and neurotrophin receptor mRNAs in primary sensory neurons of aged rats, J. Comp. Neurol. 375 (1996) 303–319.
[2] E.R. Blough, K.M. Rice, D.H. Desai, P. Wehner, G.L. Wright, Aging alters mechanical and contractile properties of the Fisher 344/Nnia X Norway/Binia rat aorta, Biogerontology 8 (2007) 303–313.
[3] S.D. Brain, T.J. Williams, H.R. Tippins, J.R. Morris, I. MacIntyre, Calcitonin gene-related peptide is a potent vasodilator, Nature 313 (1985) 54–56.
[4] M. Chauhan, P.R. Gangula, S.J. Wimalawansa, C. Yallampalli, Studies on the effects of the N-terminal domain antibodies of calcitonin receptor-like receptor and receptor activity-modifying protein 1 on calcitonin gene-related peptide induced vasorelaxation in rat uterine artery, Biol. Reprod. 70 (2004) 1658–1663.
[5] L.T. Clark, Issues in minority health: atherosclerosis and coronary heart disease in African Americans, Med. Clin. North Am. 89 (2005) 977–1001.
[6] L.T. Clark, F.E. Atat, Metabolic syndrome in African Americans: implications for preventing coronary heart disease, Clin. Cardiol. 30 (2007) 161–164.
[7] L.T. Clark, K.C. Ferdinand, J.M. Flack, J.R. Gavin 3rd, W.D. Hall, S.K. Kumanyika, J.W. Reed, E. Saunders, H.A. Valantine, K. Watson, N.K. Wenger, J.T. Wright, Coronary heart disease in African Americans, Heart Dis. 3 (2001) 97–108.
[8] Y.L. Dong, S. Vegiraju, M. Chauhan, P.R. Gangula, G.D. Hankins, L. Goodrum, C. Yallampalli, Involvement of calcitonin gene-related peptide in control of human fetoplacental vascular tone, Am. J. Physiol. Heart Circ. Physiol. 286 (2004) H230–H239.
[9] R.K. Dubey, B. Imthurn, M. Barton, E.K. Jackson, Vascular consequences of menopause and hormone therapy: importance of timing of treatment and type of estrogen, Cardiovasc. Res. 66 (2005) 295–306.
[10] R.K. Dubey, S. Oparil, B. Imthurn, E.K. Jackson, Sex hormones and hypertension, Cardiovasc. Res. 53 (2002) 688–708.
[11] M.Y. Farhat, M.C. Laving, P.W. Ram well, The vascular protective effects of estrogen, FASEB J. 10 (1996) 615–624.
[12] M.B. Fausett, M.A. Belfort, R. Nanda, G.R. Saade, Y.P. Vedernikov, The effects of sex steroids on human umbilical artery and vein, J. Soc. Gynecol. Invest. 6 (1999) 27–31.
[13] P.R. Gangula, P. Lanlua, R.D. Bukoski, S.J. Wimalawansa, C. Yallampalli, Mesenteric arterial relaxation to calcitonin gene-related peptide is increased during pregnancy and by sex steroid hormones, Biol. Reprod. 71 (2004) 1739–1745.
[14] P.R. Gangula, S.J. Wimalawansa, C. Yallampalli, Pregnancy and sex steroid hormones enhance circulating calcitonin gene-related peptide levels in rats, Hum. Reprod. 15 (2000) 949–953.
[15] P.R.R. Gangula, P. Lanlua, S.J. Wimalawansa, S.C. Supowit, D.J. DiPette, C. Yallampalli, Regulation of calcitonin gene-related peptide expression in dorsal root ganglia of rats by female sex steroid hormones, Biol. Reprod. 62 (2000) 1033–1039.
[16] P.R.R. Gangula, S.J. Wimalawansa, C. Yallampalli, Progesterone upregulates vasodilator effects of calcitonin gene-related peptide in NG-nitro-L-arginine methyl ester-induced hypertension, Am. J. Obstet. Gynecol. 176 (1997) 894–900.
[17] P.R.R. Gangula, S.J. Wimalawansa, C. Yallampalli, Sex steroid hormones enhance hypotensive effects of calcitonin gene-related peptide in aged female rats, Biol. Reprod. 67 (2002) 1881–1887.
[18] P.R.R. Gangula, H. Zhao, S.J. Wimalawansa, S.C. Supowit, D.J. DiPette, C. Yallampalli, Pregnancy and steroid hormones enhance the systemic and regional hemodynamic effects of calcitonin gene-related peptide (CGRP) in rats, Biol. Reprod. 64 (2001) 1776–1783.
[19] X. Guo, M. Razandi, A. Pedram, G. Kassab, E.R. Levin, Estrogen induces vascular wall dilation: mediation through kinase signaling to nitric oxide and estrogen receptors alpha and beta, J. Biol. Chem. 280 (2005) 19704–19710.
[20] S. Gupta, S. Mehrotra, C. Villalon, V.R. De, I. Garrelds, P. Saxena, A.M. Vandenbrink, Effects of female sex hormones on responses to CGRP, acetylcholine, and 5-HT in rat isolated arteries, Headache 47 (2007) 564–575.
[21] J.J. Holman, R.K. Craig, I. Marshall, Human alpha- and beta-CGRP and rat alpha-CGRP are coronary vasodilators in the rat, Peptides 7 (1986) 231–235.
[22] A. Ichikawa, H. Sumino, T. Ogawa, S. Ichikawa, K. Nitta, Effects of long-term transdermal hormone replacement therapy on the rennin–angiotensin–aldosterone system, plasma bradykinin levels and blood pressure in normotensive postmenopausal women, Geriatr. Gerontol. Int. 8 (2008) 259–264.
[23] V.V. Koledova, R.A. Khalil, Sex hormone replacement therapy and modulation of vascular function in cardiovascular disease, Expert Rev. Cardiovasc. Ther. 5 (2007) 777–789.

[24] S. Lamon-Fava, J.B. Barnett, M.N. Woods, C. McCormack, J.R. McNamara, E.J. Schaefer, C. Longcope, B. Rosner, S.L. Gorbach, Differences in serum sex hormone and plasma lipid levels in Caucasian and African–American premenopausal women, J. Clin. Endocrinol. Metab. 90 (2005) 4516–4520.
[25] Y. Li, Effect of age on vascular content of calcitonin gene-related peptide and mesenteric vasodilator nerve activity in the rat, Eur. J. Pharmacol. 236 (1993) 373–378.
[26] R.A. Lobo, S. Bélisle, W.T. Creasman, N.R. Frankel, N.F. Goodman, J.E. Hall, S.L. Ivey, S. Kingsberg, R. Langer, R. Lehman, D.B. McArthur, V. Montgomery-Rice, M. Notelovitz, G.S. Packin, R.W. Rebar, M. Rousseau, R.S. Schenken, D.L. Schneider, K. Sherif, S. Wysocki, Should symptomatic menopausal women be offered hormone therapy? Med Gen. Med. 8 (2006) 40.
[27] E. Lokkeggard, A.H. Andreasen, R.K. Jacobsen, L.H. Nielsen, C. Agger, O. Liddegard, Hormone therapy and risk of myocardial infarction: a national register study, Eur. Heart J. 29 (2008) 2660–2668.
[28] J.K. Lu, P.S. LaPolt, T.E. Nass, D.W. Matt, H.L. Judd, Relation of circulating estradiol and progesterone to gonadotropin secretion and estrous cyclicity in aging female rats, Endocrinology 116 (1985) 1953–1959.
[29] J.M. Manson, M.D. Sammel, E.W. Freeman, J.A. Grisso, Racial differences in sex hormone levels in women approaching the transition to menopause, Fertil. Steril. 75 (2001) 297–304.
[30] N.C. Maria, A.C. Stefan, S.G. Karin, K. Karolina, Acute dilatation to phytoestrogens and estrogen receptor subtypes expression in small arteries from women with coronary heart disease, Atherosclerosis 196 (2008) 49–58.
[31] E. Marti, S.J. Gibson, M.N. Polak, P. Facer, D.R. Springall, G. Aswegen, M. Aitchison, M. Koltzenburg, Ontogeny of peptide and amino-containing neurons in motor, sensory and autonomic regions of rat and human spinal cord, dorsal root ganglia and rat skin, J. Comp. Neurol. 266 (1987) 332–359.
[32] L.M. McLatchie, N.J. Fraser, M.J. Main, A. Wise, J. Brown, N. Thompson, R. Solari, M.G. Lee, S.M. Foord, RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor, Nature 393 (393) (1998) 333–339.
[33] S.R. Miller, L.M. Gallicchio, L.M. Lewis, J.K. Babus, P. Langenberg, H.A. Zacur, J.A. Flaws, Association between race and hot flashes in midlife women, Maturitas 54 (2006) 260–269.
[34] T. Miyauchi, Y. Tomobe, T. Ishikawa, K. Goto, Y. Sugishita, Calcitonin gene-related peptide (CGRP) induces more potent vasorelaxation in the resistance portion than in the conduit portion of mesenteric arteries in humans, Peptides 17 (1996) 877–879.
[35] V. Montgomery Rice, Hormone replacement therapy: optimizing the dose and route of administration, Drugs Aging 19 (2002) 807–817.
[36] A. Nechmad, G. Merin, H. Schwalb, D.V. Shimon, J.B. Borman, E. Milgalter, M. Mosseri, Estrogen induces nitric oxide-mediated vasodilation of human mammary arteries in vitro, Nitric Oxide 2 (1998) 460–466.
[37] H.D. Nelson, L.L. Humphrey, P. Nygren, S.M. Teutsch, J.D. Allan, Postmenopausal hormone replacement therapy: scientific review, J. Am. Med. Assoc. 288 (2002) 872–881.
[39] Neves-E-Castro, G. Samsioe, M. Doren, O. Skouby, Results from WHI and HERS II—implications for women and the prescriber of HRT, Maturitas 42 (2002) 255–258.
[40] O. Nevo, J.F. Soustiel, I. Thaler, Cerebral blood flow is increased during controlled ovarian stimulation, Am. J. Physiol. Heart Circ. Physiol. 293 (2007) H3265–H3269.
[41] M.S. Post, M.O. Verhoeven, M.J. van der Mooren, P. Kenemans, C.D. Stehouwer, T. Teerlink, Effect of hormone replacement therapy on plasma levels of the cardiovascular risk factor asymmetric dimethylarginine: a randomized, placebo-controlled 12-week study in healthy early postmenopausal women, J. Clin. Endocrinol. Metab. 88 (2003) 4221–4226.
[42] X. Qiao, R. Kristi, B.S. McConnell, R.A. Khalil, Sex steroids and vascular responses in hypertension and aging, Gend. Med. 5 (2008) S46–S64.
[43] A. Spinetti, A. Margutti, S. Bertolloni, F. Bernardi, G. BiFulco, E.C. Uberti, F. Petraglia, A.R. Genazzani, Hormonal replacement therapy affects calcitonin gene-related peptide and atrial natriuretic peptide secretion in postmenopausal women, Eur. J. Endocrinol. 137 (1997) 664–669.
[44] J.P. Stice, J.P. Eiserich, A.A. Knowlton, Role of aging versus the loss of estrogens in the reduction in vascular function in female rats, Endocrinology 150 (2009) 212–219.
[45] C. Stonier, J. Bennett, E.A. Messenger, G.M. Aber, Oestradiol-induced hypotension in spontaneously hypertensive rats: putative role for intracellular cations, sodium-potassium flux and prostanoids, Clin. Sci. 82 (1992) 389–395.
[46] T. Unemoto, H. Honda, H. Kogo, Differences in the mechanisms for relaxation of aorta induced by 17beta-estradiol or progesterone between normotensive and hypertensive rats, Eur. J. Pharmacol. 472 (2003) 119–126.
[47] A. Valentini, F. Petraglia, D. De Vita, C. Nappi, A. Margutti, E.C. degli Uberti, A.R. Genazzani, Changes of plasma calcitonin gene-related peptide levels in postmenopausal women, Am. J. Obstet. Gynecol. 175 (1996) 638–642.
[48] D. van Rossum, U.K. Hanisch, R. Quirion, Neuroanatomical localization, pharmacological characterization and functions of CGRP, related peptides and their receptors, Neurosci. Biobehav. Rev. 21 (1997) 649–678.
[49] R. Vera, M. Sánchez, M. Galisteo, I.C. Villar, R. Jimenez, A. Zarzuelo, F. Pérez-Vizcaíno, J. Duarte, Chronic administration of genistein improves endothelial dysfunction in spontaneously hyperactive rats: involvement of eNOS, caveolin and calmodulin expression and NADPH oxidase activity, Clin. Sci. 112 (2007) 183–191.
[50] A.J. Welton, M.R. Vickers, J. Kim, D. Ford, B.A. Lawton, A.H. MacLennan, S.K. Meredith, J. Martin, T.W. Meade, for the WISDOM Team, Health related quality of life after combined hormone replacement therapy: randomised controlled trial, Br. Med. J 337 (2008) a1190, doi:10.1136/bmj.a1190.
[51] M.E. Wierman, W.M. Kohrt, Vascular and metabolic effects of sex steroids: new insights into clinical trials, Reprod. Sci. 14 (2007) 300–314.
[52] S.J. Wimalawansa, Calcitonin gene-related peptide and its receptors: molecular genetics, physiology, pathophysiology and therapeutic potentials, Endocr. Rev. 17 (1996) 533–585.
[53] S.J. Wimalawansa, A.A. El-Kholy, Comparative study of distribution and biochemical characterization of brain calcitonin gene-related peptide receptors in five different species, Neuroscience 54 (1993) 513–519.
[54] X. Xu, J.C. Xiao, L.F. Luo, S. Wang, J.P. Zhang, J.J. Huang, M.L. Liu, C.G. Liu, K.Q. Xu, Y.J. Li, H.P. Song, Effects of ovariectomy and 17beta-estradiol treatment on the renin-angiotensin system, blood pressure, and endothelial ultrastructure, Int. J. Cardiol. 130 (2007) 196–204.
[55] C. Yallampalli, S.B. Kondapaka, P. Lanlua, S.J. Wimalawansa, P.R. Gangula, Female sex steroid hormones and pregnancy regulate receptors for calcitonin gene-related peptide in rat mesenteric arteries, but not in aorta, Biol. Reprod. 70 (2004) 1055–1062.