ORIGINAL RESEARCH

Loss of Female Sex Hormones Exacerbates Cerebrovascular and Cognitive Dysfunction in Aortic Banded Miniswine Through a Neuropeptide Y–Ca^{2+}-Activated Potassium Channel–Nitric Oxide Mediated Mechanism

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***Background*—**Postmenopausal women represent the largest cohort of patients with heart failure with preserved ejection fraction, and vascular dementia represents the most common form of dementia in patients with heart failure with preserved ejection fraction. Therefore, we tested the hypotheses that the combination of cardiac pressure overload (aortic banding [AB]) and the loss of female sex hormones (ovariectomy [OVX]) impairs cerebrovascular control and spatial memory.

***Methods and Results*—**Female Yucatan miniswine were separated into 4 groups (n=7 per group): (1) control, (2) AB, (3) OVX, and (4) AB-OVX. Pigs underwent OVX and AB at 7 and 8 months of age, respectively. At 14 months, cerebral blood flow velocity and spatial memory (spatial hole-board task) were lower in the OVX groups ($P<0.05$), with significant impairments in the AB-OVX group ($P<0.05$). Resting carotid artery β stiffness and vascular resistance during central hypovolemia were increased in the AB-OVX group ($P<0.05$), and blood flow recovery after central hypovolemia was reduced in both OVX groups ($P<0.05$). Isolated pial artery (pressure myography) vasoconstriction to neuropeptide Y was greatest in the AB-OVX group ($P<0.05$), and vasodilation to the Ca^{2+}-activated potassium channel α-subunit agonist NS-1619 was impaired in both AB groups ($P<0.05$). The ratio of phosphorylated endothelial nitric oxide synthase:total endothelial nitric oxide synthase was depressed and Ca^{2+}-activated potassium channel α-subunit protein was increased in AB groups ($P<0.05$).

***Conclusions*—**Mechanistically, impaired cerebral blood flow control in experimental heart failure may be the result of heightened neuropeptide Y–induced vasoconstriction along with reduced vasodilation associated with decreased Ca^{2+}-activated potassium channel function and impaired nitric oxide signaling, the effects of which are exacerbated in the absence of female sex hormones.
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Key Words: cerebral blood flow • cognition • female sex hormones • heart failure • heart-brain relationships • vascular biology • vascular cognitive impairment

Cardiogenic dementia is commonly observed in patients with heart failure with preserved ejection fraction (HFpEF). Estimates indicate up to ≈50% of hospitalized and community-dwelling patients with HFpEF experience cardiogenic dementia[1,2] and particularly vascular dementia.[3] Given that EF and cardiac output remain normal under resting conditions,[4] HFpEF-induced vascular dementia may occur independently of resting cardiac systolic dysfunction (ie, decreased pumping capacity of the heart at rest). In a translational large animal model that displays similar cardiac features as human HFpEF, we demonstrated that heart failure–induced deficits in spatial memory in male pigs coincided with increased total peripheral resistance and carotid artery vascular resistance, independent of resting cardiac systolic impairment.[5] These data suggest that cerebrovascular dysfunction may contribute to indexes of vascular dementia in this experimental model of heart failure, and support the notion that HFpEF reflects a total-body disease affecting the peripheral vasculature and the brain.[3,6] Indeed, impaired nitric oxide (NO) signaling, a hallmark feature of HFpEF,[7,8] negatively affects cerebral blood flow control,[9]

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Clinical Perspective

What Is New?

- We report that cardiac pressure overload and the loss of female sex hormones independently contribute to cerebrovascular dysfunction (mediated in part by molecular mechanisms, including neuropeptide Y signaling, Ca^{2+}-activated potassium channel function, and nitric oxide) and cognitive impairment in a translational swine model of heart failure.

What Are the Clinical Implications?

- The development of cardiogenic dementia, and by extension the pathological mechanisms involved, occurred independent of resting cardiac systolic dysfunction and other associated comorbidities.
- Cardiac pressure overload and the loss of female sex hormones independently contribute to cerebrovascular dysfunction and cognitive impairment, independent of common comorbidities, in a translational swine model of heart failure.

which may lead to cognitive dysfunction. Currently, however, whether changes in cerebral blood flow control contribute to cognitive dysfunction in HFpEF remains unknown.

The prevalence of HFpEF in women is 2 times greater than observed in men, with morbidity and mortality rates increasing with advancing age.[10,11] These sex- and age-specific effects may relate to the loss of female sex hormones after menopause. It remains unknown whether the loss of female sex hormones exacerbates potential impairments in cerebrovascular function and/or deficits in cognition in patients with HFpEF. Postmenopausal women[12] and ovariectomized (OVX) female rats[13] display augmented peripheral vasoconstriction to the sympathetic cotransmitter neuropeptide Y (NPY). Similarly, rodents with experimentally induced congestive heart failure also exhibit augmented NPY-mediated peripheral vasoconstriction.[14] At the same time, both the female sex hormone estrogen[15,16] and experimental heart failure[17,18] may interact with vascular smooth muscle ion channels, and through reductions in Ca^{2+}-activated potassium channel (BK_{Ca}) function, decrease protection against receptor-mediated vasoconstriction. Potentially, the combination of the loss of female sex hormones in conjunction with heart failure facilitates excessive NPY-induced vasoconstriction in the cerebral circulation. Collectively, these changes may contribute to pathological changes in cerebral blood flow control[19] and higher rates of cognitive dysfunction often observed in postmenopausal women,[20] women who have undergone OVX,[21] and patients with heart failure in general.[1–3]

Therefore, the purpose of this study was to examine the interaction between cardiac pressure overload–induced heart failure and the loss of female sex hormones on cerebral blood flow and cognition in female miniature swine subject to aortic banding (AB) and/or OVX. We hypothesized that the combination of cardiac pressure overload and the loss of female sex hormones reduces cerebral blood flow and increases NPY-induced vasoconstriction in isolated pial arteries. Furthermore, we hypothesized that reduced cerebral perfusion correlates with deficits in spatial memory performance.

Methods

Design

Sexually mature, intact, female Yucatan miniswine (7 months old; N=28) were separated into 4 groups (n=7 per group): (1) non-AB intact control (CON), (2) intact AB, (3) non-AB OVX, and (4) AB-OVX. Pigs underwent OVX at 7 months of age and AB at 8 months of age. OVX was performed 1 month before AB to ensure the loss of endogenous sex hormones before the initiation of pressure overload. After 5.5 months of cardiac pressure overload, cognitive testing was performed. Thereafter, cerebral and cranial blood flow control were examined in vivo. After euthanasia, 2a pial arteries were isolated and vasomotor function was assessed in vitro. All animal protocols were in accordance with the Principles for the Utilization and Care of Vertebrate Animals Used in Testing Research and Training and approved by the University of Missouri (Columbia, MO) Animal Care and Use Committee. The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure. However, similar methods have been published by our group previously,[5,22,23] and researchers may contact the corresponding author about methodological questions.

Ovariectomies

Both OVX and AB-OVX groups (7 months of age) were sedated with Telazol (tiletamine hydrochloride and zolazepam hydrochloride)/xylazine (5.0/2.25 mg/kg, respectively) and maintained under anesthesia with 3.0% isoflurane. Left and right ovaries were exposed via a midline incision. The arteriovenous complex was ligated with 0 absorbable suture, and the ovary was transected. After excision, the ovarian bursa was opened and the ovary was inspected to confirm complete ovarian removal.[24]

Aortic Banding

Heart failure was induced with AB over a period of 24 weeks, as previously reported by our laboratory,[5,22,23] in AB and AB-

OVX miniature swine at 8 months of age. The AB was placed around the ascending aorta, and a systolic transstenotic gradient of ≈70 mm Hg was achieved (73±3 and 72±3 mm Hg for AB and AB-OVX, respectively; P=0.83). The AB was set under equivalent hemodynamic conditions for all pigs. Mean arterial pressure (MAP) under anesthesia was maintained at ≈90 mm Hg (90±1 and 89±1 mm Hg for AB and AB-OVX, respectively; P=0.50) using phenylephrine (IV 1–3 μg/kg per minute), and heart rate was ≈100 beats per minute (99±5 and 109±4 beats per minute for AB and AB-OVX, respectively; P=0.14). Left ventricular brain natriuretic peptide mRNA was measured to assess heart failure, as previously reported by our laboratory.[23]

Cognitive Testing

Spatial memory was tested using a spatial hole-board recognition task (modified from Bolhuis et al[25] and previously used by our laboratory).[5] After standardization of trial performance among groups, the testing procedure consisted of 2 phases: (1) acquisition phase and (2) retention phase. The acquisition phase consisted of exposure to the spatial hole-board task for 1 180-second trial (or until all food baits were sampled) daily for 5 consecutive days. Five days after the last day of the acquisition phase, the retention phase was performed and consisted of 1 180-second exposure to the spatial hole-board task. Scores for working memory (a form of short-term memory) were calculated as the ratio of the number of first-time visits to baited bowls:the total number of visits to baited bowls. Scores for reference memory (a form of long-term memory) were calculated as the ratio of the number of visits to the baited bowls:the total number of visits to all bowls. Average memory score was calculated as the mean of working and reference memory scores.[25] Spatial maps were created for each pig's retention trial, and spatial navigation patterns were classified into 2 separate categories: (1) spatial, characterized by a high degree of accuracy or serial search pattern, with minimal revisiting, independent of accuracy (ie, sequentially check each bowl, with minimal back-tracking, until all food baits are located); and (2) nonspatial, characterized by no apparent search strategy (ie, checking bowls in what appears to be a random order) or a fixed inaccurate search strategy (ie, revisiting the same incorrect bowls). The order of testing was randomized, and experimenters T.D.O., J.A.H., and J.C.E. were blinded to the experimental group during testing and spatial navigation analyses.

Altrenogest Dosing

To account for the potentially confounding issue of changes in cardiovascular function with cyclic variations in sex hormone levels during the menstrual cycle,[26] menstrual cycles were synchronized to ensure the intact swine groups were not in estrus at the time of euthanasia. The CON and AB groups were dosed orally with the steroidal progestin, altrenogest (4.5 mL, 0.22% solution; MATRIX, Merk, New Jersey), for 14 days, followed by 12 to 15 days of nontreatment before euthanasia. MATRIX half-life is 13 hours; thus, no altrenogest was present in the animals at euthanasia.[27] Serum progesterone concentrations were measured using a chemiluminescent enzyme immunoassay (IMMULITE 1000) to verify that all experiments were performed during anestrus.

In Vivo Cardiovascular Testing

Cardiovascular testing took place over 2 sessions. In the first session, pigs were anesthetized using a mixture of Telazol/xylazine (2.5 and 1.13 mg/kg, respectively), and middle cerebral artery blood velocity was examined using transcranial Doppler ultrasonography. An inherent limitation of transcranial Doppler ultrasonography is that cerebral artery blood flow velocity must be used to estimate cerebral perfusion, because in vivo arterial diameters cannot be resolved. Briefly, a 2-MHz probe was placed on the fronto-orbital region, and the angular orientation of the probe and signal depth were adjusted to obtain an optimal velocity signal. The Doppler signal was recorded using a Powerlab data acquisition system. In the second session, anesthesia was induced with a mixture of Telazol/xylazine (5 and 2.25 mg/kg, respectively) and maintained using propofol (6–10 mg/kg per minute). At this dose, propofol does not impair cerebral autoregulation in pigs.[28,29] Pigs were placed in the supine position, and cardiac output and EF% (5F admittance-based ADVantage catheter inserted into the left ventricle through an apical incision) as well as heart rate (ECG) and MAP (fluid-filled 6F arterial catheter) were measured. Carotid artery blood flow (≈2 cm inferior to the bifurcation) was measured using Doppler ultrasonography, as previously published.[5] The Doppler audio signal was converted to a real-time analog signal by a customized Doppler audio translator,[30] and blood velocity was recorded using a Powerlab data acquisition system. Concurrent time-aligned data for heart rate, MAP, and blood velocity were extracted using Labchart 7. Carotid artery blood flow was calculated as the product of the measured blood velocity and cross-sectional area of the carotid artery, as determined from the measured diameter. Carotid artery vascular resistance was calculated as the quotient of MAP and carotid artery blood flow.[5] Resting blood flow (velocity) and vascular resistance data were averaged over an ≈30-second period. Vascular mechanics were averaged over 3 cardiac cycles and calculated, as previously described.[31]

Vena cava occlusion was performed to induce central hypovolemia, as previously described.[5,22,23] MAP and carotid artery blood flow were recorded at baseline, during balloon

inflation/vena cava occlusion, at the end of inflation, and for 60 seconds after deflation. Data during the inflation and deflation were averaged across quintiles based on time (ie, 5 12-second averages were calculated for the 60-second period after recovery from vena cava occlusion). Body temperature was maintained using heating pads and blankets throughout the entire experiment.

In Vitro Pial Arterial Function

After euthanasia, 2a pial arteries were harvested and transferred to a Plexiglas chamber filled containing ice-cold physiological saline solution (PSS: NaCl 145 mmol/L, KCl 4.7 mmol/L, $CaCl_2$ 2.0 mmol/L, and $MgSO_4$ 1.17 mmol/L, with 10 g/L albumin added) with a pH of 7.4. Thereafter, they were cannulated with 2 glass micropipettes (≈80 μmol/L) filled with PSS, warmed to 37°C, and equilibrated at an intraluminal pressure of 60 mm Hg for 1 hour. The chambers were transferred to the stage of an inverted microscope attached to a video camera, a video micrometer, and a MacLab data acquisition system. Luminal diameter and pressure were monitored continuously throughout the experiment. In total, 3 2a pial arteries were mounted for each animal. Each vessel was warmed to 37°C and equilibrated at an intraluminal pressure of 60 mm Hg for 1 hour. Vasomotor responses to NPY ($1e^{-9}$-$1e^{-6}$ mol/L) were examined in untreated (artery 1), Y1 receptor (artery 2; 30 minutes preincubation with BIBP-3226; $3e^{-7}$), and Y2 receptor antagonism (artery 3; 30 minutes preincubation with BIIE-0246; $3e^{-7}$) conditions. We also examined vasodilatory capacity of each vessel via activation of the large conductance BK_{Ca} using the BK_{Ca} α-subunit agonist NS-1619 ($1e^{-10}$-$1e^{-4}$ mol/L). All vessels exhibited similar 20% to 40% myogenic tone before examination of vasodilation (CON, 33±2%; AB, 29±3%; OVX, 30±3%; and AB-OVX, 29±2%; P≥0.41). At the end of each experiment, vessels were washed twice with Ca^{2+}-free PSS and exposed to $1e^{-4}$ mol/L sodium nitroprusside, and the maximal lumen diameter was recorded at 60 mm Hg. There were no differences in passive pial artery diameters among groups (CON, 321±34 μm; AB, 305±12 μm; OVX, 289±25 μm; and AB-OVX, 279±32 μm; P≥0.30). Percentage vasoconstriction was calculated as the quotient of Δ response−baseline and the maximal passive diameter in Ca^{2+}-free PSS+sodium nitroprusside, multiplied by 100. Percentage possible dilation was calculated as the quotient of Δ response−baseline and Δ maximal passive diameter in Ca^{2+}-free PSS+sodium nitroprusside−baseline, multiplied by 100.

Protein expression was determined according to previous methods used in this laboratory.[32] Briefly, polyvinylidene difluoride membranes were blocked in a 5% nonfat milk–Tris-buffered saline–Tween 20 solution and incubated overnight at 4°C with a primary antibody against the NPY-Y1 receptor (55 kDa, 1:1000), BK_{Ca} α-subunit (110–130 kDa; 1:1500), endothelial NO synthase (eNOS; 140 kDa; 1:1000), phosphorylated eNOS (Ser 1177; 140 kDa; 1:500), and β-actin (42 kDa; 1:2000). Membranes were incubated with a horseradish peroxidase–conjugated anti-rabbit or anti-mouse secondary antibody in a 5% nonfat milk–Tris-buffered saline–Tween 20 solution. Blots for all proteins were then incubated in Luminata Forte Western HRP substrate visualization reagent, and a Kodak image station was used to visualize and quantify protein band densities. No differences in β-actin levels (loading control) were observed among groups; therefore, absolute data are presented relative to the CON group.

Statistical Analysis

Memory scores, baseline hemodynamics, and immunoblotting data were analyzed using a 2-way ANOVA (AB by OVX). Hemodynamic variables during vena cava occlusion and pial artery myography data were analyzed using a 3-way ANOVA (AB by OVX by time point or dose). The Pearson correlation coefficients for systolic blood pressure, diastolic blood pressure, middle cerebral artery blood flow velocity, and average memory score were determined. Significant interactions were explored using a post hoc Student Newman-Keuls test. Unless stated, there were no differences in pairwise comparisons. On the basis of the results of the 2-way ANOVA and to increase the total number of observations per group, categorical data from the spatial navigation were pooled according to intervention (AB and OVX) and analyzed using a Fisher exact test (2×2 contingency table). Only the pooled data stratified by OVX status were significant and are presented. All data are presented as mean±SEM, and significance is reported at the P<0.05 level.

Results

Physical and Cardiac Parameters

Body mass was not significantly different (P≥0.13), but cardiac pressure overload increased the heart weight:body weight ratio (main effect of AB; P<0.05; all data presented in Table 1). Systolic, diastolic, and MAPs were not significantly different between groups (P≥0.15). The loss of female sex hormones increased total peripheral resistance (main effect of OVX; P<0.05). Cardiac output and EF% (≥45%)[33] were normal in all groups, despite EF% being statistically lower in the AB-OVX group compared with the AB and OVX groups (AB×OVX interaction; P<0.05). Cardiac pressure overload increased left ventricular mRNA for brain natriuretic peptide levels relative to CON (main effect of AB; P<0.05). The combination of normal EF% and increased left ventricular brain natriuretic

Table 1. Body Mass, Hemodynamic, Cardiac, and Sex Hormone Parameters

Parameters	CON	AB	OVX	AB-OVX
Mass, kg	43±1	39±3	40±1	38±2
HW:BW, g/kg	4.3±0.2	5.3±0.3*	4.1±0.1	4.9±0.2*
SBP, mm Hg	100±7	96±7	114±4	101±5
DBP, mm Hg	86±6	83±8	99±3	89±4
MAP, mm Hg	91±6	87±8	104±4	93±4
TPR, mm Hg/L per minute	17±2	14±1	21±2†	19±2†
CO, L/min	6.2±0.9	6.3±0.7	5.3±0.3	5.1±0.4
EF%	64±2	69±2	68±2	58±3‡
LV BNP mRNA	1.0±0.4	6.1±2.7*	0.8±0.4	7.0±1.8*
Uterus weight, g	436±28	368±25	42±4†	36±3†

Data are given as mean±SEM. AB indicates aortic banded; BW, body weight; CO, cardiac output; CON, control; DBP, diastolic blood pressure; EF, ejection fraction; HW, heart weight; LV BNP, left ventricular brain natriuretic peptide; MAP, mean arterial pressure; OVX, ovariectomized; SBP, systolic blood pressure; and TPR, total peripheral resistance.
*Main effect of AB.
†Main effect of OVX ($P<0.05$).
‡AB×OVX interaction, AB-OVX vs AB and OVX.

peptide suggests heart failure with compensated resting systolic function (ie, HFpEF). Overall, the AB-OVX group displayed signs of heart failure in combination with elevated total peripheral resistance, suggesting the combination of cardiac pressure overload and decreased female sex hormones impairs peripheral and cardiac function more than either intervention alone. OVX decreased uterine weight (main effect of OVX; $P<0.05$), as expected with the loss of female sex hormones after OVX. Serum progesterone levels at the time of euthanasia for CON (16±4 ng/mL) and AB (16±4 ng/mL) animals were higher than levels corresponding with estrous (>0.5 ng/mL),[34,35] confirming intact animals were not in estrous at the time of terminal experiments.

Cognition and In Vivo Hemodynamics

The loss of female sex hormones resulted in decreased reference and working memory scores (main effect of OVX; $P<0.05$; Figure 1A and 1B; raw data presented in Table 2). Pairwise comparisons revealed that working memory scores were most reduced in AB-OVX compared with AB animals ($P<0.05$; Figure 1B). The Fisher exact test revealed that the loss of female sex hormones increased use of nonspatial search strategies ($P<0.05$; Figure 1C and 1D). The loss of female sex hormones also decreased middle cerebral artery blood flow velocity (main effect of OVX; $P<0.05$; Figure 1E), and again this effect was more pronounced in AB-OVX compared with AB animals (post hoc, $P<0.05$; Figure 1E). Middle cerebral blood flow velocity and average memory scores (reference+working memory scores combined) were positively correlated ($P<0.01$; Figure 1F). Systolic ($R=0.33$; $P=0.12$) and diastolic ($R=0.38$; $P=0.07$) blood pressures were not significantly correlated with average memory scores.

At baseline, before vena cava occlusion, there were no differences in pulse pressure ($P\geq0.30$; Figure 2A) or carotid artery systolic or diastolic diameters (average systolic diameter for all pigs, 4.9±0.1 mm; average diastolic diameter for all pigs, 4.6±0.1 mm; $P\geq0.18$). However, both cardiac pressure overload and the loss of female sex hormones decreased carotid artery strain (main effect of AB and OVX; $P<0.05$; Figure 2B) and increased carotid artery β stiffness (main effect of AB and OVX; $P<0.05$; Figure 2C). Pairwise comparisons revealed carotid artery β stiffness was greater in AB-OVX versus either AB or OVX alone ($P<0.05$; Figure 2C). There were no differences in baseline carotid artery blood flow, MAP, or vascular resistance ($P\geq0.17$). Both cardiac pressure overload and the loss of female sex hormones decreased carotid artery blood flow during and in recovery from vena cava occlusion (main effect of AB and OVX; $P<0.01$; Figure 3A). The loss of female sex hormones caused a greater impairment in blood flow recovery, as indicated by decreased percentage blood flow recovery relative to baseline at 1 minute after the end of occlusion (main effect of OVX; $P<0.05$; Figure 3B). MAP was lowest in the AB group compared with CON and AB-OVX animals during and after vena cava occlusion (AB×OVX interaction; $P<0.01$; Figure 3C). Relative to baseline, blood pressure recovered fully in all groups by 1 minute after occlusion ($P\geq0.67$; Figure 3D). During vena cava occlusion, carotid vascular resistance was greater in OVX compared with CON animals, and in AB-OVX compared with AB and OVX groups (AB×OVX interaction; $P<0.05$; Figure 3E). After vena cava occlusion, carotid vascular resistance was increased by both cardiac pressure overload and the loss of female sex hormones (main effect of AB and OVX; $P<0.05$; Figure 3E). During the recovery phase, the loss of female sex hormones was associated with increased percentage carotid vascular resistance relative to baseline (main effect of OVX; $P<0.05$; Figure 3F), and this effect was greatest in AB-OVX compared with AB animals (post hoc, $P<0.05$; Figure 3F).

In Vitro Pial Artery Function and Protein Levels

In isolated pial arteries, vasoconstriction to NPY was greater in AB-OVX compared with the AB and OVX groups, and also increased in the AB and OVX compared with CON group (AB×OVX interaction; $P<0.05$; Figure 4A). Blockade of the Y1, but not Y2, receptor reduced pial artery vasoconstriction in the AB, OVX, and AB-OVX groups (main effect of AB and OVX; $P<0.05$; Figure 4B), indicating augmented NPY-induced

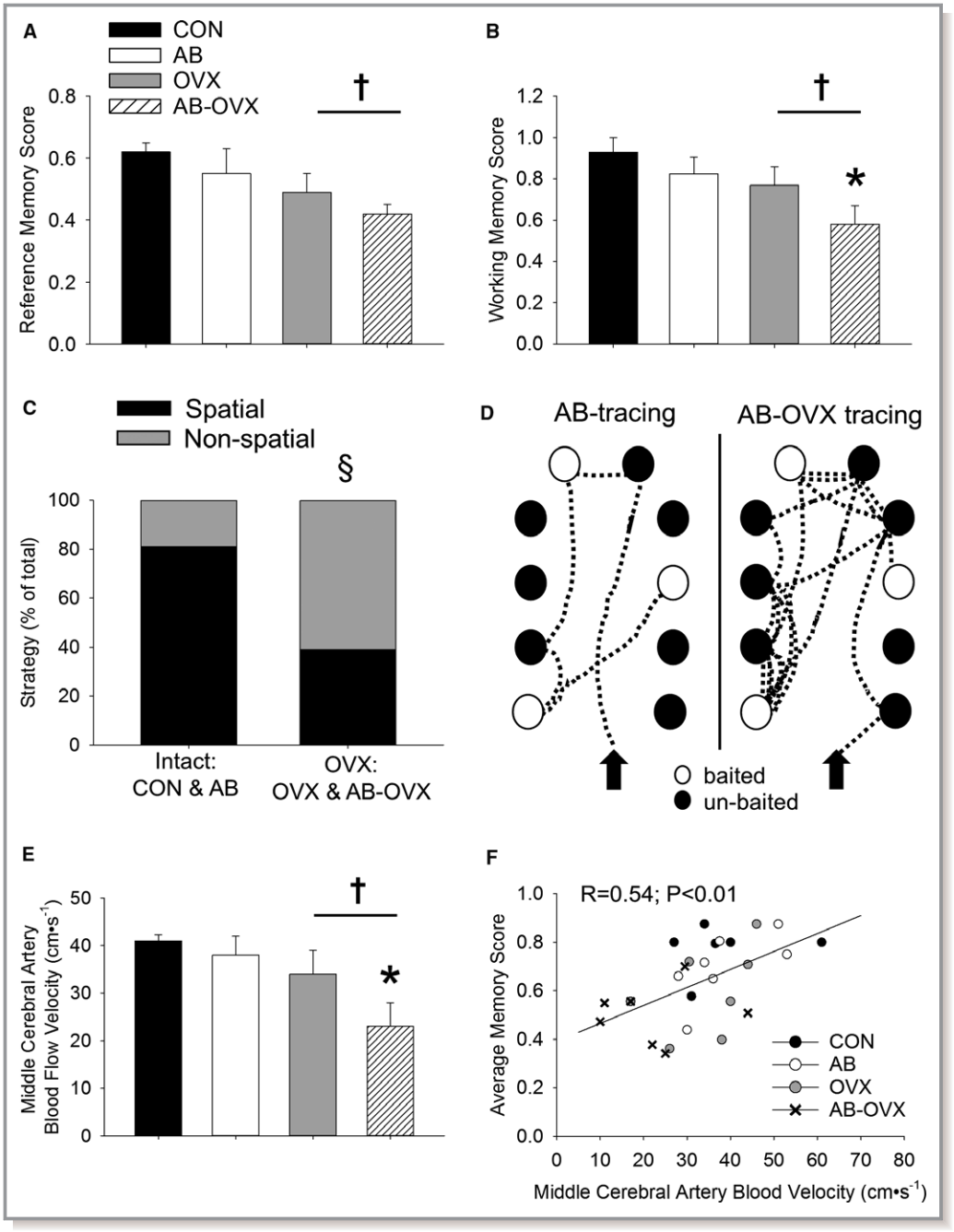

Figure 1. Reference (A) and working (B) memory scores, spatial navigation strategies used (C), example tracings (D), middle cerebral artery blood flow velocity (E), and the relationship between middle cerebral blood flow velocity and average memory scores (F). AB indicates aortic banded; CON, control; and OVX, ovariectomized. *Post hoc vs AB ($P<0.05$). †Main effect of OVX and increased reliance on nonspatial search strategies ($P<0.05$).

vasoconstriction was acting through NPY-Y1 receptor signaling. Furthermore, Y1 blockade was more pronounced in AB-OVX compared with AB animals (post hoc, $P<0.05$; Figure 4B). Although pial artery sensitivity to NPY was increased by both cardiac pressure overload and the loss of female sex hormones, pial artery Y1 receptor protein levels were similar among groups relative to CON (CON, 1.0±0.2; AB, 1.1±0.3; OVX, 0.8±0.1; AB-OVX, 1.06±0.4 normalized densitometry; $P\geq0.53$). Cardiac pressure overload decreased pial artery vasodilation to the BK_{Ca} α-subunit agonist NS-1619 (main effect of AB; $P<0.01$; Figure 4C), but increased pial artery BK_{Ca} α-subunit protein levels relative to CON (main effect of AB; $P<0.05$; Figure 4D). The ratio of phosphorylated eNOS:-total eNOS was decreased by cardiac pressure overload (main

Table 2. Retention Trial Spatial Hole-Board Visits Required to Locate 3 Food Baits

Type of Visit	CON	AB	OVX	AB-OVX
Total visits required	6±1	8±2	11±2*	14±3*†
Incorrect visits	3±1	4±2	6±2*	9±2*
Incorrect revisits	0±0	1±2	2±1	3±2
Correct revisits	1±0	1±1	2±1*	3±1*†

Data are given as mean±SEM. AB indicates aortic banded; CON, control; and OVX, ovariectomized.
*Main effect of OVX ($P<0.05$).
†Post hoc vs AB ($P<0.05$).

effect of AB; $P<0.05$; Figure 4E). Representative Western blots for all proteins are shown in Figure 4F.

Discussion

The current study implicates reduced cerebral perfusion in the development of cardiogenic dementia in a translational porcine model of heart failure, as reflected in the positive association between middle cerebral blood flow velocity and cognition, occurring independent of clinical deficits in resting cardiac systolic function. In the context of cerebrovascular dysfunction in experimental postmenopausal cardiogenic dementia (ie, AB-OVX group), results of this study reveal that impairments in blood flow control in vivo coincided with carotid arterial stiffening, heightened vasoconstriction to the sympathetic cotransmitter NPY, depressed vasodilation to the BK_{Ca} channel α-subunit agonist NS-1619, and decreased phosphorylated eNOS:total eNOS ratio in isolated 2a pial arteries. The results indicate that both cardiac pressure overload and the loss of female sex hormones independently contributed to changes in indexes of cerebrovascular control observed in vivo and in vitro. However, only the combination of AB and OVX produced deficits in resting cerebral blood flow velocity and cognition, highlighting the possibility that postmenopausal women with increased cardiac afterload may be at a greater risk of cardiogenic dementia, independent of age.

In the current study, the loss of female sex hormones combined with cardiac pressure overload exacerbated impairments in cerebrovascular and cognitive function, indicated by decreased cerebral blood flow velocity and working memory (which was associated with decreased reliance on spatial navigation strategies) in the AB-OVX group. This finding reconciles independent observations that the loss of female sex hormones, naturally or via OVX,[20,21] and HFpEF are associated with cognitive dysfunction, particularly vascular dementia.[1–3] In addition, the AB-OVX group displayed increased carotid artery stiffening and vascular resistance during/after central hypovolemia. Relative to previously reported data from male AB pigs,[5] AB had a similar impact on indexes of carotid arterial stiffening and cognition in the AB-OVX group, but changes in these outcomes were less pronounced in the intact AB group from the current study. Collectively, this raises the possibility that female sex hormones help protect against arterial stiffening and cognitive decline in the setting of experimental cardiac pressure

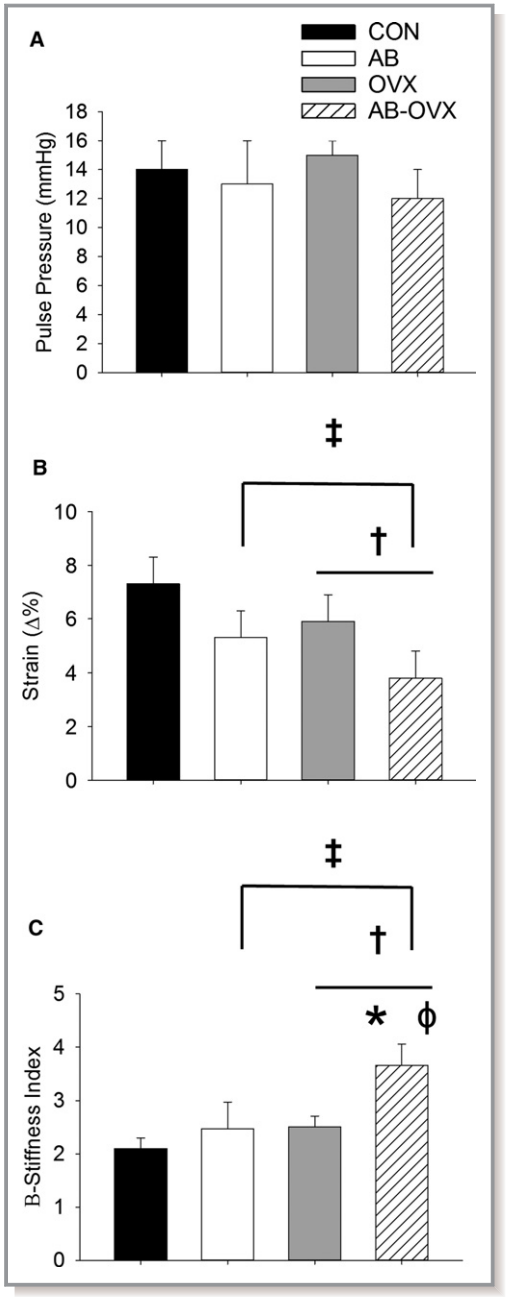

Figure 2. Carotid artery pulse pressure (A), arterial strain (B), and the β-stiffness index (C) at baseline. AB indicates aortic banded; CON, control; and OVX, ovariectomized. *Post hoc vs AB. †Main effect of OVX ($P<0.05$). ‡Main effect of AB ($P<0.05$). ϕPost hoc vs OVX.

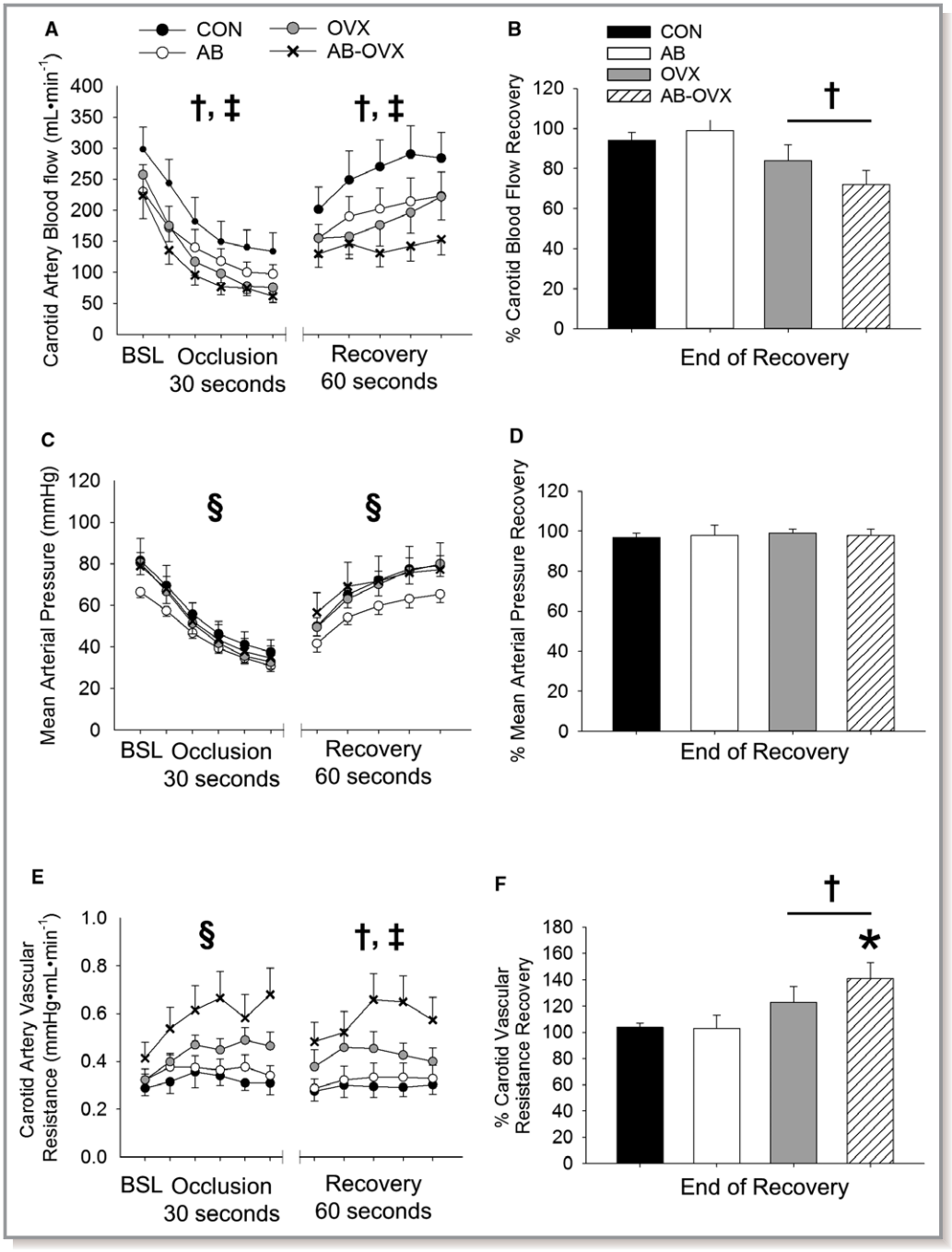

Figure 3. Carotid artery blood flow (A) and percentage flow recovery (B), mean arterial pressure (C) and percentage pressure recovery (D), and carotid artery vascular resistance (E) and percentage vascular resistance recovery (F) at baseline (BSL) and in recovery from central hypovolemia. AB indicates aortic banded; CON, control; and OVX, ovariectomized. *Post hoc vs AB ($P<0.05$). †Main effect of OVX ($P<0.05$). ‡Main effect of AB ($P<0.05$). §Interaction effect AB×OVX ($P<0.05$).

overload. Humans with HFpEF commonly display carotid arterial stiffening[36]; however, whether this directly influences cerebral blood flow control and the increased rates of vascular dementia in HFpEF remains unknown.

Mechanistically, our data suggest that augmented vasoconstriction to NPY in isolated pial arteries may play a significant role in the cognitive impairment observed in AB-OVX animals. Pial arteries account for ≈50% of total cerebral vascular resistance.[37] After middle cerebral artery occlusion, a single short-term intracarotid injection of NPY reduces cerebral blood flow recovery and increases cerebral vascular resistance from the onset of reperfusion for up to 2 hours in

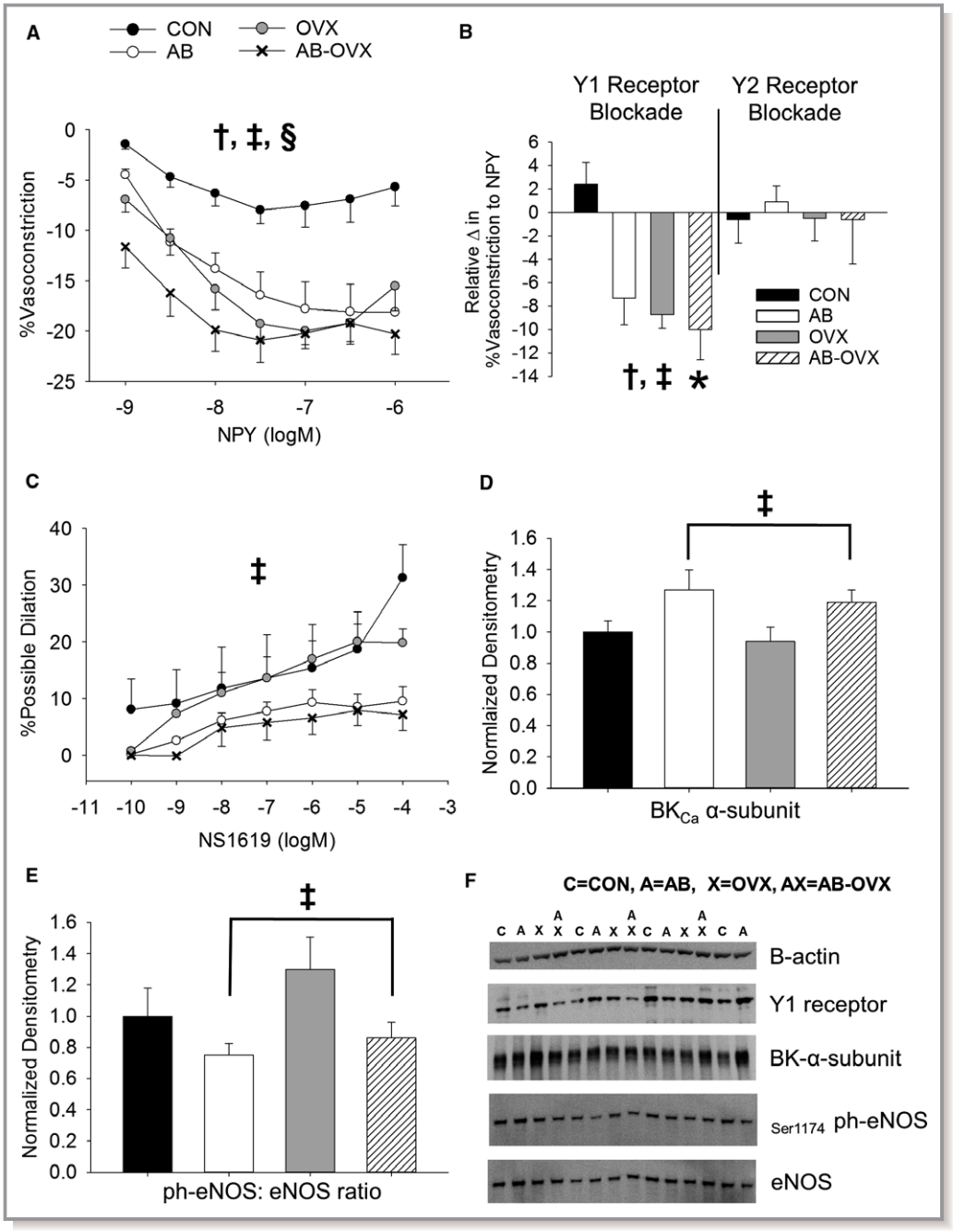

Figure 4. Neuropeptide Y (NPY)–induced vasoconstriction (A), relative change in NPY-induced vasoconstriction during Y1 and Y2 receptor blockade (B), NS-1619–induced vasodilation (C), Ca^{2+}-activated potassium (BK_{Ca}) α-subunit protein level relative to control (CON; D), phosphorylated endothelial nitric oxide synthase ($_{Ser1174}$ph-eNOS):total eNOS ratio relative to CON (E), and representative Western blot images (F). AB indicates aortic banded; and OVX, ovariectomized. *Post hoc vs AB (P<0.05). †Main effect of OVX (P<0.05). ‡Main effect of AB (P<0.05). §Interaction effect AB×OVX (P<0.05).

rats.[38] Therefore, it seems possible that increased vasoconstrictor responses to NPY contribute to reduced cerebral blood flow and increased cerebral vascular resistance in the basal state and during sympathoexcitation.[39–41] Patients with HFpEF commonly exhibit augmented sympathetic nervous system activity,[42] the effects of which, on cerebral blood flow control, have not been studied rigorously.

Depressed NO signaling (tonic or endothelium-dependent NPY induced),[43] indicated by a reduced eNOS phosphorylation status, and reduced ability of the BK_{Ca} channel to counteract vasoconstriction represent endothelium-dependent and endothelium-independent mechanisms that may have contributed to augmented NPY-induced pial artery vasoconstriction observed in pigs exposed to cardiac pressure

overload. Irrespective of sex hormone status, pigs exposed to cardiac pressure overload exhibited reduced BK_{Ca} channel–dependent vasodilation, despite increases in pial artery BK_{Ca} channel α-subunit protein levels. Evidence suggests that BK_{Ca} channels serve as an endogenous buffer to counteract increases in arterial tone in response to elevations in vascular smooth muscle intracellular Ca^{2+}. High blood pressure may increase BK_{Ca} channel protein content in rat cerebral microcirculation.[44] Metabolic dysfunction[45] also appears to increase BK_{Ca} channel protein content. In line with the current study, data from the coronary vasculature of obese pigs with metabolic syndrome show a similar pattern of impaired BK_{Ca} channel–dependent vasodilation in parallel with increased overall expression of the BK_{Ca} channel itself.[45] Indeed, decreased BK_{Ca} α-channel–induced vasodilation may impair the ability of vascular smooth muscle to buffer against excessive vasoconstrictor stimuli.[17,18,44,45]

The underlying cause of the discrepancy in channel expression and vasomotor control observed in the current study remains unknown, but may be linked to function of the BK_{Ca} channel β1-subunit. For example, reduced NO-mediated β1-subunit trafficking to the plasma membrane may influence kinetics, calcium sensitivity, and BK_{Ca} channel activity in response to pharmacological modulators.[16,46] In support of this line of reasoning, irrespective of sex hormone status, cardiac pressure overload also reduced the ratio of phosphorylated eNOS:total eNOS in pial arteries, indicating a potential reduction in NO signaling. Given that NO exerts direct effects on BK_{Ca} channel activation,[15,16,46] and both NO and BK_{Ca} channel activation induce cerebral vasodilation,[9,47] BK_{Ca} channel levels may have been increased to partially compensate for reduced eNOS phosphorylation status and subsequent lower NO signaling. In total, the combination of increased cerebrovascular sensitivity to the vasoconstrictive effects of NPY and decreased vasodilatory influence of NO and the BK_{Ca} channel may represent an integrative mechanism involved in cerebrovascular and cognitive dysfunction in this setting of experimental heart failure.

Although HFpEF is more often seen in older individuals,[10,11] it is impractical to age pigs to true senescence given their relatively long lifespan (≈15 years) for experimental research.[48] Thus, we recognize a limitation of the model is an inability to study HFpEF in a context of natural aging, along with a gradual loss of sex hormones. However, the strength of this swine model lies in its translational relevance to human hemodynamic and cardiovascular function, in addition to similarities inherent to the brain (ie, pig brains are gyrencephalic and contain >60% white matter).[49–51] Overall, the strengths of the swine model from both a translational hemodynamic and a cognitive perspective outweigh its weakness as a true model of senescence.

Perspective

Compared with patients with heart failure without cognitive impairment, patients with heart failure with undocumented cognitive impairment experience increased rates of readmission to the hospital and increased rates of mortality.[2] Hospitalized patients with heart failure commonly present with cognitive impairment, yet diagnosis occurs infrequently,[2] highlighting a need to better understand risk factors, identify biomarkers, and improve screening for dementia in this patient population. Resting cardiac systolic impairment and other comorbidities, including aging, obesity, and type 2 diabetes mellitus, are common risk factors of cardiogenic dementia.[1,2,52–54] The present findings revealed that independent of these well-recognized factors, the combination of pressure overload–induced heart failure and loss of female sex hormones may expose patients to an increased risk of cerebrovascular and cognitive dysfunction. Evidence suggests female sex hormones, and particularly estrogen, reduce NPY-induced vasoconstriction, in part by a hormonal-mediated downregulation of the Y1 receptor.[13] Furthermore, estrogen has been implicated in increased BK_{Ca} channel opening (via the β1-subunit)[55] and increased NO signaling (via upregulation of eNOS).[9,19,56] Interestingly, these mechanisms did not fully explain the perceived benefit of female sex hormones, or alternatively the detrimental effect of OVX, on cerebrovascular and cognitive function, given the in vitro cerebral microvascular impairments present in both intact and AB-OVX animals. Beyond potential species-related differences, these observations provide the foundation for future studies to elucidate the role of female sex hormones in the prevention of vascular dementia in the setting of heart failure. Given the widespread accessibility of cognitive testing (ie, Montreal Cognitive Assessment) and vascular imaging tools (ie, Doppler ultrasonography), validating these concepts clinically is feasible and will help identify at-risk populations and streamline efforts towards appropriate documentation and treatment of patients with heart failure who have dementia.

Conclusion

This study provided evidence that the loss of female sex hormones and cardiac pressure overload independently alter cerebrovascular function and cognition in a porcine model of experimental heart failure. More important, the development of cardiogenic dementia, and by extension the pathological mechanisms involved, occurred independent of resting cardiac systolic dysfunction and other associated comorbidities (ie, age, obesity, and type 2 diabetes mellitus). Specifically, these data implicate impairments in indexes of cerebrovascular blood flow control, mediated in part by molecular

mechanisms, including NPY signaling, BK_{Ca} channel function, and NO, may play a critical role in the pathological features.

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Disclosures

None.

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