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Norepinephrine activity, as measured by MHPG, is associated with menopausal hot flashes

S. L. Dormire and R. Bongiovanni*

The University of Texas at Austin, School of Nursing, Austin, Texas

*Louis Stokes VMAC at Brecksville, Brecksville, Ohio, USA

Abstract

Objectives—Baseline norepinephrine levels, as measured by a metabolite (plasma 3-methoxy-4-hydroxyphenolglycol, MHPG), have been reported to increase in women who experience hot flashes. However, norepinephrine is also discharged in a counter-regulatory attempt to increase brain glucose as normal daily variations occur. The purpose of this analysis is to examine the relationship between hot flush frequency and MHPG under conditions of experimental glucose manipulation.

Methods—A repeated-measures experimental design study was conducted with ten postmenopausal women taking hormone therapy between the ages of 38 and 55 years. In a 30-h experimental protocol, participants received normal saline and 20% glucose intravenous infusions on sequential days and were monitored for hot flashes and blood glucose changes. MHPG levels were evaluated before and after each experimental condition as a biomarker of norepinephrine activity.

Results—Although hot flush frequency was significantly different between infusion periods, mean MHPG levels were not statistically different (normal saline period, 3.1 ng/ml; glucose infusion, 3.2 ng/ml). No distinct patterns of MHPG change were found in this sample.

Conclusions—In this study, there was no consistent pattern of MHPG increase or decrease in the women experiencing hot flashes.

Keywords

HOT FLUSHES; MHPG; GLUCOSE; NOREPINEPHRINE; MENOPAUSE

INTRODUCTION

Evidence indicates that norepinephrine has a role in the physiology of menopausal hot flashes. One of the current models of hot flashes proposes a transient decrease of the thermoregulatory set point, causing a central autonomic response. This central response leads to an increase in heart rate and release of cortisol, mineralocorticoids, β -endorphin and luteinizing hormone¹. In related animal research, Brück and Zeisberger² found that injection of norepinephrine near the rat hypothalamus causes peripheral vasodilation, heat loss and a decrease in core temperature, similar to the events of a hot flush. In humans, however, brain norepinephrine cannot be measured in plasma³. Instead, plasma 3-methoxy-4-hydroxyphenolglycol (MHPG),

a metabolite of brain norepinephrine, provides a measure of brain norepinephrine level. In previous studies, MHPG levels have been used to provide an indirect measure of norepinephrine activity associated with the menopausal hot flush. Freedman and Woodward⁴ found increased baseline MHPG levels in symptomatic women and MHPG further increased after hot flushes.

The purpose of this paper is to examine changes in MHPG levels when hot flush incidence is altered by experimental manipulation of blood glucose in symptomatic postmenopausal women. In the previous studies, baseline MHPG levels were increased in symptomatic women and further increased following hot flush episodes. Therefore, we hypothesized that MHPG levels would be elevated following fasting blood glucose conditions, when hot flush frequency was expected to increase, and MHPG levels would be unchanged when blood glucose was experimentally elevated and hot flush frequency theoretically would be diminished. In this study, comparison of MHPG levels before and after each experimental condition provided a biomarker of norepinephrine activity.

METHODS

Design

An experimental cross-over design was used to examine the objectives of this study. Participants were admitted to the General Clinical Research Center of a large Midwestern university for a 30-h intensive blood sampling protocol that included 3.5-h experimental phases on two sequential mornings and one non-fasting observational phase between them. During the two morning experimental phases, each participant was exposed to randomly ordered intravenous infusions of glucose and normal saline.

Participants

Postmenopausal women on hormone therapy for management of menopausal hot flushes and willing to withdraw from hormone therapy for the purposes of this study were the identified population for this study. Inclusion criteria included postmenopausal status as defined by the absence of a menstrual period for 12 months with demonstrated postmenopausal levels of estradiol and follicle stimulating hormone (FSH), no current medical or psychiatric illness, and no current medication usage. To control for factors known to alter the incidence of hot flushes, women who smoked and those with a body mass index greater than 31 kg/m² or less than 20 kg/m² were excluded. Twelve postmenopausal women were recruited. This sample size provided adequate power to detect mean group frequency differences of 2.5, assuming a standard deviation of 3 and a two-sided test of the null hypothesis of the paired differences in hot flush frequency between the experimental conditions.

Study volunteers meeting inclusion criteria were also screened for general health through completion of a history and physical examination by a nurse practitioner. A complete blood count and differential, in addition to a renal profile, were completed to assure absence of conditions that would affect individual health in the study. In addition, potential participants were screened for diabetes with a glycosylated hemoglobin, fasting blood glucose, and 2-h glucose tolerance test. Postmenopausal status was confirmed through analysis of estradiol and FSH levels. Data from two participants were eliminated from the final analysis because of elevated estradiol levels. Following the screening visit, participants were instructed to stop hormone therapy and maintain a daily hot flush diary for 7-10 days. When hot flushes were experienced at least four times per day in a consecutive 3-day period, participants were scheduled for admission to the General Clinical Research Center for data collection.

Measures

MHPG—MHPG samples were collected at the beginning and end of each experimental period. Using standard procedure, 10 ml of venous blood was collected in a vacutainer lavender tube (K2EDTA). After the blood collection, the tubes were gently inverted and immediately placed on ice. After a 10-min post-collection waiting period, the blood sample was centrifuged at approximately 2400 g for 10 min. The plasma was collected and transferred to individually labeled plastic tubes for storage at -80°C. Samples were maintained at -80°C until analyzed.

Skin conductance—Skin conductance is a measure of the resistance associated with moisture on the skin. Sweat gland activity, such as occurs during a hot flush, decreases skin resistance and increases the skin's ability to conduct current passing through it. Decreasing skin resistance is an established objective measure of the menopausal hot flush⁵. In this study, hot flush incidence was objectively measured through continuous monitoring using the Biolog® Skin Conductance Monitor (UFI Model 3991/1 SCL, UFI, Morro Bay, CA, USA). The skin conductance level is digitally displayed on the monitor screen and the data are saved on a computer disc in the monitor. The data are downloaded using a product-specific card reader. Data from the Biolog® are graphically displayed for analysis using flush customized software (DPS v.1.5®, UFI, Morro Bay, CA, USA). A standard criterion of increase in skin conductance of greater than a 2 μ mhos in less than 30 s is considered a valid hot flush⁶. Also, participants noted perceived hot flushes by pressing a button on the monitor to event mark on the tracing.

Blood glucose level—For each blood glucose sample, 0.5 ml of blood was drawn from a catheter placed in the dominant forearm and flushed with heparinized saline. The 0.5-ml sample of venous blood was centrifuged for 20 s and five drops of unhemolyzed plasma were pipetted into the Beckman Glucose Analyzer⁷.

Procedures

Skin conductance monitoring was initiated at admission using the standard application procedures^{8,9}. For blood sampling, a heparinized catheter was placed in one forearm, with a second catheter placed in the antecubital space of the other arm for infusion of fluids during the experimental periods of the study. At the start of each experimental period, a blood sample was collected and processed for MHPG analysis.

Following completion of baseline data collection, the intravenous infusion assigned was initiated using a standard pump set at the initial rate of 100 ml/h. Participants received an infusion of normal saline or 20% glucose randomly ordered during the experimental periods. Using infusion principles of the glucose clamp technique¹⁰⁻¹², a variable rate infusion of 20% dextrose maintained the blood glucose level above 130 mg/dl. Samples for blood glucose were drawn every 5 min during a 30-min titration period. Normal saline infusion rate was randomly adjusted during the titration to maintain a sham since participants were blinded to infusion type. Following each titration period, blood glucose was sampled every 15 min for the 3-h experimental period. Intravenous flow was adjusted to maintain blood glucose above 130 mg/dl during the 20% dextrose infusion, and random rate changes were maintained during the normal saline infusion. At the end of each 3.5-h experimental period, a sample was again collected for MHPG, providing four such samples per participant.

Plasma MHPG determination

Plasma samples were kept at -80°C until the day of analysis. Samples were thawed and then centrifuged at 3000 g for 10 min at 4°C. Aliquots of 500 μ l volume of the plasma supernatant were subsequently used for analysis. The method of Ojala-Karlson was used for the sample concentration.

The Bond-Elut extraction columns were activated in the Vac-Elut manifold by passing 1 ml of methanol, then 1 ml of water and finally 2 ml of OSA (octane sulfonic acid, 1 mmol/l). Subsequently, a sample aliquot was added to the column and allowed to slowly pass through. The column was washed with 0.3 ml water. MHPG was then eluted with 0.5 ml of 30% methanol. Afterwards, ethyl acetate (6 ml) was added to each sample eluent, followed by vigorous mixing for 30 s. The mixture was centrifuged (3000 g) for 5 min. The organic phase was brought to dryness with a gentle stream of nitrogen. The residue was dissolved in 150 μ l of HPLC mobile phase and 20 μ l injected on column.

The instrument consisted of a solvent delivery system (Model 6000), a fluorescence detector (Model 470, excitation/emission set at 275/315 $\pm$ 18 nm) with a manual fixed loop (20 μ l) injector (all from Water Associates, Milford, MA, USA). The separation was accomplished with a reverse phase column (C18, 10 cm $\times$ 4.6 mm) and a mobile phase consisting of an aqueous (in mmol/l: 10 citric acid, 20 sodium acetate, 0.4-0.5 octane sulfonic acid)/methanol (95/5, v/v), final pH 3.0. The integration (peak height) was accomplished with a chromatography software package (Justice Laboratory Software, Denville, NJ, USA). Routinely, a linear working concentration range, 0-10.0 ng/ml ($R^2 > 0.998$) was utilized for MHPG quantitation. Additionally, a laboratory pooled human serum control was used daily along with analysis of the clinical samples. The control intra-assay results were 3.66 $\pm$ 0.12 ng/ml (mean $\pm$ standard deviation (SD), $n = 16$).

RESULTS

Clinical characteristics

This protocol was approved by the Institutional Review Board and all participants gave informed consent. All participants were in good health, as assessed by a medical history and a physical examination by a nurse practitioner. Concentrations of follicle stimulating hormone (mean 67.9, SD 41.9 mIU/ml) and estradiol (mean 15.2, SD 5.1 pg/ml) were in the expected ranges for postmenopausal women and all laboratory indices of general health were within normal limits. All were medication-free other than postmenopausal hormone therapy. The average participant in this study was 48.6 years old, well educated with 15.4 years of schooling, 5 years past menopause, and had a body mass index of 25.4 kg/m². Eight were naturally menopausal and four were surgically menopausal. There were only two significant differences by type of menopause: surgically menopausal women were slightly younger and had more hot flushes during the experimental periods.

Hot flush frequency

The total sample of participants experienced three hot flushes during the glucose infusion and 23 hot flushes during the normal saline infusion period. Hot flush frequency between the two testing periods differed significantly ($t = -2.4$, d.f. = 9, $p = 0.04$). The magnitude of the effect was moderate ($d = -0.699$). Hot flush frequency was muted when blood glucose was experimentally sustained (130-140 mg/dl) and frequency was increased under conditions of fasting blood glucose levels.

MHPG change

Although hot flush frequency differed significantly between the two infusion periods, mean MHPG levels at the end of the infusion periods were not statistically different (normal saline period, 3.1 ng/ml; glucose infusion, 3.2 ng/ml). Also, no clear patterns of change in MHPG were found within individuals for infusion periods with and without hot flushes.

Several of the participants in this study did not experience objectively measured hot flushes during either experimental period, although they reported subjective experiences of hot flushes.

Therefore, we sought to evaluate the differences between those who had objectively measured hot flushes and those who had not. Among the five women who experienced hot flushes during the normal saline infusion, three experienced slight increases in MHPG (mean 0.24 ng/ml). One participant experienced seven hot flushes during this period but also experienced a net decrease in MHPG by 0.37 ng/ml. The net change in MHPG levels during glucose administration (three hot flushes experienced) in this same subset of women actually increased to a greater extent than during the normal saline administration, when 23 hot flushes were experienced.

Patterns of MHPG change between the hot flush and no-hot flush groups during each experimental period were also examined (see Table 1). There were no distinct patterns of MHPG changes in this sample. For the hot flush group, the greatest increase in MHPG levels was observed in the slight increase during the glucose administration period, when few hot flushes were experienced. The no-hot flush group experienced the greatest mean change in MHPG, with a decrease of -0.26 ng/ml.

DISCUSSION

In this report, the relationship between menopausal hot flush frequency and MHPG levels was examined under experimental conditions of blood glucose manipulation in symptomatic women. MHPG provides an indirect measure of norepinephrine and has been reported to increase during hot flush episodes. However, the mechanism of this increase is not well understood.

The Impaired Glucose Delivery Model of Vasomotor Symptoms¹³ may provide some insight into the mechanism of norepinephrine release. The model suggests that hot flushes result from changes in brain glucose delivery involving central and peripheral supply processes. The synchrony of three central coupling processes (neurometabolic, neurovascular and neurobarrier) maintains glucose levels for neuron metabolic functioning. These interrelated processes include neuron activation, metabolic activation, blood flow, and glucose transport across the blood-brain barrier¹⁴. The peripheral processes stimulating central processes include blood flow and blood glucose levels. The interrelationship of these processes serves to protect the metabolic needs of the brain since only a 2-min supply of glucose is maintained centrally¹⁵.

As glucose demands increase with neuron activation, the neurobarrier system is stimulated to increase availability of glucose transporter at the blood-brain barrier. Glucose transporter 1 (GLUT1) is the carrier protein in the plasma membranes of endothelial cells of brain interstitium, whose function is to move glucose molecules into the brain via facilitated diffusion¹⁶. Neurobarrier coupling signals increased glucose transport at the blood-brain barrier by increasing both the rate of transcription of GLUT1 messenger RNA and the number of GLUT1 molecules in the blood-brain barrier. In addition, vasodilation proportional to glucose need is stimulated via the neurovascular coupling system. This mechanism serves to adapt glucose and oxygen delivery in response to neuronal metabolic needs¹⁷. The number of functional GLUT1 transporters increases by virtue of the increased surface area of perfused capillaries responding to the increased blood flow¹⁸.

Changes in neuron glucose demand result in stimulation of these coupling processes. In addition, changes in peripheral glucose supply stimulate these coupling processes. Elevated peripheral glucose levels diminish neurobarrier and neurovascular coupling processes, while declining peripheral blood glucose levels stimulate these processes.

Estrogen plays a role in this system by facilitating the production of GLUT1 when the demand for glucose transport increases, either under conditions of brain activation or low blood

glucose levels. In the presence of estradiol, glucose transport at the blood-brain barrier increases by up to 40%¹⁹. By augmenting GLUT1 in the cerebral cortex²⁰, estrogen enables rapid response to changing glucose needs.

The Impaired Glucose Delivery Model of Vasomotor Symptoms proposes that the hot flush is the result of an exaggerated neurovascular coupling system response, as estrogen decline diminishes the ability to increase GLUT1 as needed. That is, when estrogen levels decrease in menopause, the responsiveness of GLUT1 production to the central demands for increased glucose transport (either because of increased neuronal activity or lowered blood glucose levels) is constrained. The hot flush is seen as a counter-regulatory neurovascular response, causing vasodilation with resultant increased blood flow to aid delivery of glucose and oxygen delivery to meet the metabolic needs associated with neuronal activation. Norepinephrine is also normally discharged in a counter-regulatory attempt to increase brain glucose as normal variations in availability occur in feeding and fasting behavior.

Freedman²¹ measured both vanillylmandelic acid (VMA) and MHPG before and after hot flushes in 14 symptomatic women. He found that plasma MHPG increased significantly between pre- and post-flush samples, while VMA remained the same. Freedman and Woodward⁴ found that basal levels of MHPG were elevated in women who were symptomatic of hot flushes, when compared with symptom-free women and levels increased further during both induced and spontaneous hot flushes. Building on this evidence, we measured MHPG in a study examining the relationship between hot flushes and experimentally controlled blood glucose levels. MHPG levels provided biomarkers of norepinephrine activity before and after each of two experimental conditions, one designed to induce hot flushes and one designed to preclude them. One hypothesis explored in this study was that baseline plasma MHPG levels would increase following periods of increased hot flush frequency, as found in previous studies⁴.

However, analysis of the changes in MHPG levels in this sample did not support increasing baseline levels related to hot flush experiences. In the total sample, there was no consistent pattern of MHPG increase or decrease in the women experiencing hot flushes. Among the five women who experienced a total of 23 hot flushes during the normal saline infusion, the average change in MHPG was an increase of 0.05 ng/ml. In this same subset of women, the net change in MHPG was an increase of 0.11 ng/ml during the glucose administration in which only three hot flushes were experienced. In the subset of women who did not experience any objectively identified hot flushes during either experimental condition, the net change in MHPG was -0.26 ng/ml during the normal saline administration and 0.03 ng/ml during the glucose infusion period. The greatest individual increase in MHPG was noted during the glucose infusion in a participant who experienced no hot flushes (1.05 ng/ml). This same participant experienced six hot flushes during the normal saline period, with a 0.25 ng/ml increase in MHPG.

These data indicate that MHPG may not be an effective biomarker of the hot flush experience during menopause. Freedman and Woodward⁴ measured MHPG at 60-min intervals as well as before, during and after each perceived hot flush. For both baseline levels as well as immediate experience levels, MHPG was increased for women who had hot flushes. Since MHPG was not measured during individual hot flushes in the current study, no observations can be made regarding the increase Freedman and Woodward observed during hot flushes. However, increased baseline levels of MHPG as a result of menopausal hot flushes were not observed in these data.

MHPG levels are associated with a variety of factors. MHPG levels increase with stress²² and decrease with depression²³. Therefore, MHPG changes are not specific to hot flushes, thus making it a less than adequate biomarker. Since neither Freedman and Woodward⁴ nor the

current study screened their small samples for psychological stress or depression, their MHGP findings could have been influenced by these factors.

In addition, measurement of peripheral norepinephrine metabolites, specifically MHPG, may reflect various sources and distributions of norepinephrine. Kopin and colleagues²⁴ found that approximately 50% of the free MHPG is metabolized peripherally to VMA. This would indicate that fluctuations in peripheral VMA could warp measurement of plasma MHPG.

Further, the women in the present sample were withdrawn from hormone therapy to allow the return of hot flashes. It is possible that the 7-10-day washout period was not adequate, affecting the MHPG response. More women may have experienced hot flashes had there been a 30-day withdrawal period. However, given the similarity of results between the subset of women who did not experience hot flashes and those who did, it is unlikely that results would have been dramatically different.

With these results, additional examination of MHPG is needed as a measure of the brain norepinephrine changes associated with the menopausal hot flush. Peripheral metabolism of the norepinephrine metabolites may mute the effectiveness of MHPG as an indirect measure when plasma evaluation is not directly correlated with measurable hot flashes.

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References

1. Kronenberg F. Hot flashes: epidemiology and physiology. *Ann N Y Acad Sci* 1990;592:52–86. [PubMed: 2197954]
2. Brück, K.; Zeisberger, E. Adaptive changes in thermoregulation and their neuropharmacological basis. In: Schönbaum, E.; Lomax, P., editors. *Thermoregulation: Physiology and Biochemistry*. Pergamon; New York: 1990. p. 255-307.
3. Lambert GW, Kaye DM, Vaz M, et al. Regional origins of 3-methoxy-4-hydroxyphenylglycol in plasma: effects of chronic sympathetic nervous activation and denervation, and acute reflex sympathetic stimulation. *J Auton Nerv Syst* 1995;55:169–78. [PubMed: 8801267]
4. Freedman, RR.; Woodward, S. Elevated α -adrenergic responsiveness in menopausal hot flashes: pharmacologic and biochemical studies. In: Schönbaum, E.; Lomax, P., editors. *Thermoregulation: The Pathophysiological Basis of Clinical Disorders*. Karger; Basel, Switzerland: 1992. p. 6-9.
5. Freedman, RR. Menopausal hot flashes. In: Lobo, RA.; Kelsey, J.; Marcus, R., editors. *Menopause: Biology and Pathobiology*. Academic Press; San Diego: 2000. p. 215-27.
6. Molnar GW. Body temperature during menopausal hot flashes. *J Appl Physiol* 1975;38:499–503. [PubMed: 1150563]
7. Beckman Instruments Inc.. *Beckman Glucose Analyzer 2 Operating Manual*. Author; Brea, CA: 1977.
8. Dormire SL, Carpenter JA. An alternative to Unibase®/glycol as an effective non-hydrating electrolyte medium for measurement of skin conductance activity. *Psychophysiology* 2002;39:423–6. [PubMed: 12212634]
9. Carpenter JS, Andrykowski MA, Freedman RR, Munn R. Feasibility and psychometrics of an ambulatory hot flash monitoring device. *Menopause* 1999;6:209–15. [PubMed: 10486790]
10. Fanelli CG, Dence CS, Markham J, et al. Blood-to-brain glucose transport and cerebral glucose metabolism are not reduced in poorly controlled type 1 diabetes. *Diabetes* 1998;47:1444–50. [PubMed: 9726233]
11. Elahi D. In praise of the hyperglycemic clamp: a method for assessment of beta-cell sensitivity and insulin resistance. *Diabetes Care* 1996;19:278–86. [PubMed: 8742583]

12. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979;237:E214–33. [PubMed: 382871]
13. Dormire SL. The potential role of glucose transport changes in hot flash physiology: a hypothesis. *Biol Res Nurs*. 2008in press
14. Leybaert L. Neurobarrier coupling in the brain: a partner of neurovascular and neurometabolic coupling? *J Cereb Blood Flow Metab* 2005;25:2–16. [PubMed: 15678108]
15. Chih CP, Roberts EL. Energy substrates for neurons during neural activity: a critical review of the astrocyte-neuron lactate shuttle hypothesis. *J Cereb Blood Flow Metab* 2003;23:1263–81. [PubMed: 14600433]
16. Pardridge WM. Advances in cell biology of blood-brain barrier transport. *Semin Cell Biol* 1991;2:419–26. [PubMed: 1813031]
17. Pellerin L, Magistretti PJ. How to balance the brain energy budget while spending glucose differently. *J Physiol* 2001;546:325. [PubMed: 12527720]
18. Davson H, Purvis CA, Segal MB. Measurement of resistance to flow of cerebrospinal fluid. *J Physiol* 1969;292:62–64P.
19. Shi J, Simpkins JW. 17 β -Estradiol modulation of glucose transporter 1 (GLUT 1) expression in blood-brain barrier. *Am J Physiol Endocrinol Metab* 1997;272:E1016–22.
20. Cheng CM, Cohen M, Wang J, Bondy CA. Estrogen augments glucose transporter and IGF1 expression in primate cerebral cortex. *FASEB J* 2001;15:907–15. [PubMed: 11292650]
21. Freedman RR. Biochemical, metabolic, and vascular mechanisms in menopausal hot flushes. *Fertil Steril* 1998;70:1–6. [PubMed: 9660412]
22. Eisenhofer G, Kopin IJ, Goldstein DS. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacol Rev* 2004;56:331–49. [PubMed: 15317907]
23. Klimek V, Stockmeier C, Overhoiser J, et al. Reduced levels of norepinephrine transporters in the locus coeruleus in major depression. *Arch Gen Psychiatry* 2001;58:821–7. [PubMed: 11545664]
24. Kopin, LJ.; Blombery, P.; Ebert, MH., et al. Disposition and metabolism of MHPG-CD₃ in humans: plasma MHPG as the principal pathway of norepinephrine metabolism and as an important determinant of CSF levels of MHPG. In: Usdin, E., editor. *Frontiers in Biochemical and Pharmacological Research in Depression*. Raven Press; New York: 1984. p. 57-68.

Table 1
Net MHPG change and hot flush frequency in each experimental period

Subject	Normal saline infusion			Glucose infusion		
	Hot flush frequency	MHPG		Hot flush frequency	MHPG	
		Net change	Direction		Net change	Direction
F	7	-0.37	↓	0	0.22	↑
I	6	0.25	↑	0	1.05	↑
B	5	0.27	↑	2	-0.56	↓
E	3	-0.12	↓	0	-0.62	↓
J	2	0.21	↑	1	0.48	↑
Mean		0.05			0.11	
A	0	-0.76	↓	0	-0.58	↓
C	0	-0.37	↓	0	-0.24	↓
D	0	-0.05	↓	0	0.16	↑
G	0	0.01	↔	0	0.21	↑
H	0	-0.15	↓	0	0.62	↑
Mean		-0.26			.03	

MHPG, plasma 3-methoxy-4-hydroxyphenolglycol