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Effects of estrogen and progestin on the CO_2 sensitivity of hemispheric cerebral blood volume

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Abstract

Objective: High CO_2 sensitivity is one of the major characteristics of the cerebrovascular bed. It has been shown to be influenced by many differrent factors (eg, sex hormones).

Design: The effect of ovariectomy and subsequent female sexual hormone treatment on the steady-state hemispheric cerebral blood volume and CO_2 responsiveness of the hemispheric blood vessels was studied on anesthetized, ventilated, normotensive, normoxic rats. Cerebral blood volume was measured with Tomita's photoelectric method with Sandor's modification.

Results: Steady-state cerebral blood volume values in ovariectomized rats did not differ from those found in control animals. The CO_2 responsiveness of hemispheric blood vessels was higher in ovariectomized and progestin-treated, but not estrogen-treated, animals compared with controls.

Conclusions: Our results demonstrate that the CO_2 sensitivity of the hemispheric vessels is sex hormone dependent. Estrogen and progestin treatment have opposite effects on this cerebral circulatory parameter.

Key Words: Ovariectomy – Estrogen – Progesterone – Cerebral blood volume – CO_2 sensitivity.

The influence of the sex steroid hormones on the central nervous system and especially the cerebrovascular bed is controversial.[1,2] Estrogen decreases and testosterone increases cerebral vascular tone. Cerebrovascular inflammation is suppressed by estrogen but increased by testosterone and progesterone. Estrogen has important protective effects on endothelial cells.[2] Sex steroids seem capable of influencing cerebral blood flow (CBF). Results from experimental animal studies do not provide evidence of resting cerebral blood volume (CBV) being influenced by changes in estrogen status.

The value of CBV may provide basic information about the actual condition of the combined arterial, capillary, and venous compartments of the cerebrovascular bed. Clinically CBV (together with the volume of the brain parenchyma and the cerebrospinal fluid) is one of the three main factors that determine intracranial pressure. Similar to other major characteristics of cerebral vessels, high CO_2 sensitivity has been shown to be influenced by a variety of factors. However, we found no experimental evidence in the literature of the involvement of female sex steroids on CBV in either normocapnic or hypercapnic conditions.

The aim of the present study was to investigate (1) the consequences of ovariectomy and hormone treatments in a resting state on normocapnic CBV and (2) the CO_2 responsiveness of the hemispheric blood vessels at different female sex hormone levels.

METHODS

Animals and materials

Experiments were carried out in 41 adult, sexually matured, virgin, female Sprague-Dawley rats weighing 200 to 240 g at the beginning of the study. The animals were treated in accordance with the European Communities Council Directive (86/609/EEC), and the experimental protocol was approved by the Ethics Committee for Animal Research at Semmelweis University (36/1999/Bp. FÁÉEÁ). In the control rat group (n = 10), a sham operation (laparotomy) was performed, and vehicle treatments were given (sunflower oil and normal saline). In another group of animals (n = 31), bilateral ovariectomy was performed under intraperitoneal anesthesia (Nembutal 40 mg/kg IP; Phylaxia-Sanofi, Budapest, Hungary) under sterile conditions. One group of the ovariectomized rats (n = 10) received intramuscular estrogen treatment (OVX + E): estradiol propionate 450 μg/kg per week. Another group of the ovariectomized rats (n = 9) received intramuscular progestin treatment (OVX + P): medroxyprogesterone acetate 15 mg/kg once

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every 2 weeks. The third group of ovariectomized rats (n = 6) received combined estrogen and progestin treatment (OVX + C): estradiol propionate 450 μg/kg per week and medroxyprogesterone acetate 15 mg/kg once every 2 weeks IM. The fourth group of ovariectomized animals (n = 6) was given only the vehicles (sunflower oil and normal saline) of the sex hormones (OVX). The estradiol propionate (Biogal, Debrecen, Hungary) was freshly prepared in sunflower oil (0.9 mg/mL), and the medroxyprogesterone acetate (Depo-Provera, Upjohn, Puurs, Belgium) was dissolved in normal saline (30 mg/mL). These treatments were administered for 4 weeks after the operation. In earlier studies with the same protocol, active hormone treatments resulted in nearly physiologic hormone levels.[3,4]

Experimental procedure

After 4 weeks of treatment, the animals were anaesthetized with IP urethane (1.3 g/kg ethylcarbamate; Sigma, St. Louis, MO), artificially ventilated (Harvard Apparatus dual-phase control pump, South Natick, MA), and their body temperature was kept constant at 37°C with a controlled heating pad. Cannulas were inserted in both femoral arteries (to measure blood pressure and to draw blood samples for blood gas measurements) and in the left femoral vein (for drug administration: urethane for preserving anesthesia and heparin [Sigma] in a dose of 200 U/kg to prevent blood clotting).

To measure CBV, the head of the rat was secured in a stereotaxic head holder. The CBV was measured by Tomita's photoelectric method with Sandor's modification.[5,6] Through a hole (1.3 mm in diameter) in the skull, a miniaturized light source (a tungsten lamp 1 mm wide and 1.5 mm long [Hamai Electric Co, Tokyo, Japan]) was positioned between the two hemispheres and was fixed with dental cement. Photodiode (SBC-55 silicon blue photodiode, Sharp Electric Co, Tokyo, Japan) was attached to the outside of the skull to the internal lamina of the parietal bone and secured with light-absorbent dental cement. Assuming that the light intensity, the distance between the lamp and the photodiode, and the light extinction caused by the brain tissue remain unchanged during the experiment, the light intensity changes with the changing blood content of the transluminated brain tissue (one of the two hemispheres of the cerebrum) and can be quantified for hemispheric blood volume changes. CBV values were expressed in volume %.

Measurement of CBV

The hemispheric CBV was recorded continuously under normocapnic (at 36-38 mm Hg $PaCO_2$), hypercapnic (50-65 mm Hg $PaCO_2$), and hypocapnic (25-29 mm Hg) conditions. Hypercapnia was produced by 5% CO_2 gas inhalation, and hypocapnia was obtained by increasing the rate of the respiration. The measurements at each $PaCO_2$ level were performed after a period of at least 10 minutes at the desired $PaCO_2$ level to ensure that a new steady state was attained after each $PaCO_2$ change. From this continuous recording, a 2-minute period (during the new steady-state condition after each $PaCO_2$ change) was used with 20-Hz sampling frequency to calculate the corresponding CBVs. There was an approximate period of 25 to 30 minutes between each consecutive $PaCO_2$ level alteration.

Blood gas analysis, histologic control of the brain

The arterial blood gas values (pO_2 and pH) were kept constant in a normal physiologic range throughout all experiments by adjusting the respiratory rate and volume. Arterial blood gases were measured with a blood gas analyzer (ABL-300, Radiometer, Copenhagen, Denmark).

Animals were euthanized by anesthetic overdose. The original study design placed 10 animals in each group (50 animals all together). At the end of all experimental procedures, all the brains were removed to evaluate the possibility of local tissue damage. Brains with signs of lamp-induced local hemorrhage were not included in the study. This was the only reason for exclusion from the study.

Data analysis

All values are mean ± SEM. Physiologic parameters obtained from the different groups in normocapnic conditions were compared by one-way analysis of variance and with the Newman-Keuls post hoc test. To determine CO_2 sensitivity, statistical comparisons were performed between the initial CBV values and those obtained at different CO_2 levels within each of the five different groups (repeated-measures analysis of variance). P less than 0.05 was considered as the limit of statistical significance.

RESULTS

Steady-state hemispheric CBV in the five experimental groups

Steady-state CBV did not differ statistically ($P = 0.98$) in the ovariectomized animal group (CBV = 4.84 ± 0.07 volume %, n = 6) compared with the sham-operated control rats (CBV = 4.87 ± 0.06 volume %, n = 10). The steady-state CBV of the estrogen-treated (CBV = 4.85 ± 0.12 volume %, n = 10), the progesterone-treated (CBV = 4.96 ± 0.15 volume %, n = 9), and those animals treated with a combination (estrogen + progestin) (CBV = 4.91 ± 0.13 volume %, n = 6) did not differ statistically ($P = 0.90$, $P = 0.87$, and $P = 0.82$, respectively) from the control group (Fig. 1).

CO_2 sensitivity of hemispheric CBV after ovariectomy and different hormone treatments

In the control sham-operated group (n = 10), hemispheric CBF did not change significantly either under hypercapnic (CBV = 5.26 ± 0.21 volume %, $P = 0.10$) or hypocapnic (CBV = 4.44 ± 0.29 volume %, $P = 0.17$) conditions compared with the normocapnic steady-state CBV (CBV = 4.87 ± 0.06 volume %) (Fig. 1).

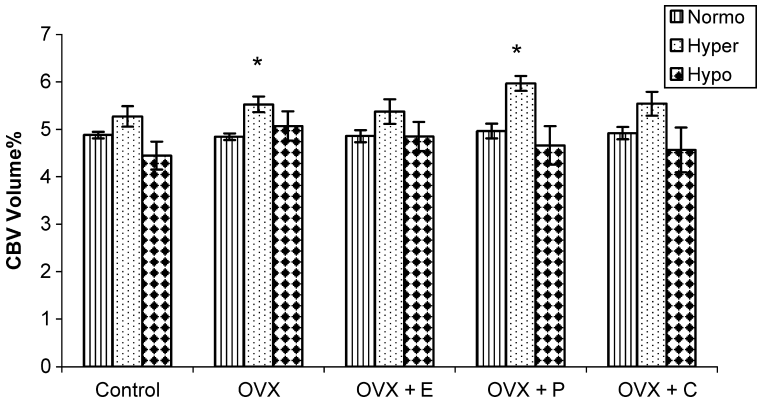

FIG. 1. Cerebral blood volume (CBV) of the five experimental groups: control, sham operated (n = 10); OVX, ovariectomized (n = 6); OVX + E, ovariectomized + estrogen-treated (n = 10); OVX + P, ovariectomized + progestin-treated (n = 9); and OVX + C, ovariectomized + estrogen + progestin treated (n = 6) in steady state (normo) at hypercapnic (hyper) and hypocapnic (hypo) conditions. Data are presented as mean ± SEM. *$P \leq 0.05$: compared with the steady-state CBV value within the same experimental group.

In the OVX group (n = 6), CBV increased significantly during hypercapnia (CBV = 5.52 ± 0.16 volume %, $P = 0.003$) compared with the normocapnic steady-state CBV (CBV = 4.84 ± 0.07 volume %). Conversely, the hypocapnic CBV (CBV = 5.07 ± 0.3 volume %, $P = 0.48$) did not differ from the normocapnic CBV value (Fig. 1).

In the OVX + E group of animals (n = 10), similar to the control group, the CBV did not change during hypercapnia (CBV = 5.37 ± 0.26 volume %, $P = 0.09$) or hypocapnia (CBV = 4.87 ± 0.3 volume %, $P = 0.98$) compared with the steady-state normocapnic CBV (CBV = 4.85 ± 0.12 volume %) (Fig. 1).

In the OVX + P group (n = 9), similar to the ovariectomized group, CBV increased significantly during hypercapnia (CBV = 5.96 ± 0.15 volume % $P = 0.0004$) compared with the normocapnic steady-state CBV (CBV = 4.96 ± 0.15 volume %). The hypocapnic CBV (CBV = 4.65 ± 0.4 volume %, $P = 0.49$) did not differ from the normocapnic CBV (Fig. 1).

In the combined (estrogen + progestin) hormone-treated group of animals (OVX + C, n = 6), the hemispheric CBF did not change significantly either under hypercapnic (CBV = 5.53 ± 0.25 volume % $P = 0.054$) or under hypocapnic (CBV = 4.6 ± 0.47 volume %) conditions compared with the steady-state normocapnic CBV (CBV = 4.91 ± 0.13 volume %, $P = 0.48$) (Fig. 1).

Circulatory and blood gas alterations after ovariectomy and sex hormone treatments

Systemic mean arterial pressures (MAPs) were as follows: OVX, MAP = 96 ± 4 mm Hg; OVX + E, MAP = 107 ± 4 mm Hg; OVX + P, MAP = 90 ± 2 mm Hg; and OVX + C, MAP = 99 ± 5 mm Hg. These values did not differ from those of the sham-operated controls (MAP = 99 ± 4 mm Hg) (Table 1).

Blood hemoglobin level did not differ statistically in the OVX and OVX + P groups compared with the control group. Blood hemoglobin levels in the OVX + E and OVX + C groups were lower than those in the control group. The hemoglobin level did not change significantly during the whole measurement procedure within each experimental group. The value of hemoglobin was checked at each different $PaCO_2$ level in our experiment.

Blood gas values (pO_2, pH) of the five experimental groups were kept in the physiologic range at the beginning of the protocol. We ensured normoxic (pO_2 = 93 ± 4-110 ± 2 mm Hg) conditions during the whole experimental procedure (Table 1).

DISCUSSION

Our experiments were the first to demonstrate that regulation of hemispheric CBV in a steady-state condition does not depend on female sex hormones. Our results suggest that ovariectomy does not result in significant change in CBV

TABLE 1. *The systemic mean arterial pressure (MAP) and blood gas values of the sham-operated (control), ovariectomized (OVX), ovarietomized + estrogen-treated (OVX + E), ovarietomized + progesterone-treated (OVX + P), and ovariectomized + combined (OVX + C) hormone-treated animal groups*

Group	MAP mm Hg	Hemoglobin (g/L)	pO_2 (mm Hg)	pCO_2 (mm Hg)	pH
Steady-state condition					
Control (n = 10)	99 ± 4	17.72 ± 0.42	103.3 ± 5.4	37.4 ± 0.4	7.36 ± 0.01
OVX (n = 6)	96 ± 4	18.16 ± 0.29	99.2 ± 3.6	37.7 ± 0.7	7.37 ± 0.01
OVX + E (n = 10)	107 ± 4	15.97 ± 0.38[a]	104.7 ± 6.2	38.3 ± 0.4	7.35 ± 0.01
OVX + P (n = 9	90 ± 2	18.58 ± 0.18	93.3 ± 3.71	38.2 ± 0.6	7.39 ± 0.01
OVX + C (n = 6)	99 ± 5	14.83 ± 0.34[a]	110.2 ± 2.01	37.2 ± 0.2	7.38 ± 0.01

Data are mean ± SEM.

[a]$P \leq 0.05$ compared with the control animal group. Blood gas values (pO_2, pH) of the five experimental groups were kept in the physiologic range at the beginning of the protocol. We ensured normoxic (pO_2 = 93 ± 4-110 ± 2 mm Hg) conditions during the whole experimental procedure. The hemoglobin concentration of the OVX and OVX + C animal groups were significantly smaller compared with the control animals.

under normocapnic steady-state conditions. In contrast, this study showed two different interactions between sex hormones and the regulation of CBV after alteration of $PaCO_2$. Ovariectomy seemed to result in significantly higher CBV during hypercapnia. In the estrogen and the combined hormone treatment groups, CBV was similar to the control group and significantly lower than in the ovariectomized animals. Conversely, there were no differences in CBV during hypocapnic conditions between the groups.

In our experiments the changes in arterial PaO_2, pH, and MAP were monitored and kept within the same physiologic range in the five different experimental animal groups. Consequently, these factors could not induce any differences in CBV between the experimental animal groups.

Hemoglobin content is an important factor when CBV is determined by Tomita's photoelectric method: increased red blood cell count is coupled with increased hemoglobin content and results in increased light extinction.

Steadiness of hemoglobin level during CBV measurement in the same animals is essential at different $PaCO_2$ levels. Hemoglobin level did not change significantly during the whole experimental procedure of our study and remained constant within each of the experimental groups.

We do not know exactly what is responsible for the differences in hemoglobin content in the different groups. It is certainly not blood loss caused by ovariectomy because the hemoglobin content was the same in the control group and the OVX group. We can hypothesize that the hormone treatment played a role in these differences, but progestin treatment in the OVX + P group does not support this hypothesis. Hemoglobin content was lower in the OVX + E and OVX+ C groups than that in the control and OVX groups. Therefore, one may suspect that it is estrogen that may be causing such changes. This is, however, very unlikely, and there are no available data in the literature that would suggest that female sex hormone treatment, either estrogen or progestin, results in a decreased hemoglobin level in rats. The question of whether hemoglobin itself plays a role in the tolerance of hypercapnia cannot be easily answered. It is well known, however, that CO_2 reacts directly (with a reversible reaction) with the hemoglobin molecules to form carbaminohemoglobin and participates in the transport of CO_2. This mechanism may provide a transport of a maximum 20% of the total quantity of CO_2.

The circulating sex steroid hormones estrogen, progesterone, and testosterone can alter both physiologic and pathophysiologic function of the cerebral circulation.[7] The two female sex hormones have more clinical relevance because of the hormone therapy widely used in the postmenopausal population some years ago. Estrogen is currently an area of controversy, ie, whether it has beneficial or detrimental effects on the risk and outcome of stroke.[8,9] Estrogen has important protective effects on endothelial function and vascular reactivity.[10] Beyond the physiologic effects of sex hormones, identification of sex hormone–dependent brain areas also seemed important.

Despite its importance, CBV is usually an underestimated parameter in cerebrovascular studies; however, its constancy during systemic arterial pressure changes (cerebral autoregulation) is an important regulation goal of cerebral circulation. In contrast, findings on the mechanisms that provide constancy of CBV are still scarce and incomplete.

In a previous study we demonstrated that nitric oxide (NO) participates in the regulation of CBV. In steady-state conditions, the total CBV could be significantly reduced by blocking the L-arginine-NO system with L-ω-nitro-L-arginine methylesther.[11] In our study the regulation of hemispheric CBV under steady-state conditions proved to be independent of female sex hormone status; normocapnic steady-state CBV was similar in the different groups.

In our previous studies we also demonstrated that the regulation of regional CBF might be sex hormone dependent in the hypothalamus.[12] Ovariectomy resulted in a significant reduction in steady-state blood flow and caused a downward shift of the lower autoregulatory threshold in the hypothalamus of the rats. Estrogen treatment prevented the ovariectomy-induced decrease of regional blood flow and shifted the lower autoregulatory threshold back to the control level. In contrast, progesterone treatment of the ovariectomized rats failed to reestablish the diminished resting blood flow, but similar to estrogen, this treatment elevated the autoregulatory threshold to the level found in control animals.[12]

Data on the possible effects of progestin on cerebrovascular circulation are scarce and incomplete in the literature. Because progesterone receptors are found in peripheral vascular tissue and cultured vascular cells, it is possible that progesterone receptors are also expressed in cerebral vessels.[13,14] There are no data in the literature on the possible effect of progestin on the reactivity of cerebral blood vessels. Acute intraperitoneally administered progesterone caused pial arterial vasodilatation, whereas estrogen failed to demonstrate this change.[15,16] Progestins do not influence the level of cerebrovascular endothelial NO synthase protein in animals.[17] During postmenopausal hormone treatment, vasodilation was found in the middle cerebral artery and in the common carotid artery.[18] Most of the studies,[19-21] but not all,[22] suggest that progesterone and its analogs seem to be neuroprotective in ischemic stroke models.

Fluctuations in endocrine status may have a global impact on cerebral circulation and can also cause regional alterations in the blood flow of specific brain regions. Data from various research groups conflict in part.[23-26] Memory testing in a positron emission tomography study showed a greater increase in cortical blood flow in estrogen-treated participants than in control participants.[27] Many,[24,27] but not all,[28] clinical studies indicate that estrogen increases CBF (measured by laser Doppler or computed tomography). CBF in women also changes during pregnancy[29] and over the course of the menstrual cycle.[24,30] Conversely, estrogen treatment in women with Alzheimer's disease was not associated with changes in cerebral perfusion.[31]

High CO_2 sensitivity, one of the major characteristics of cerebral and spinal cord vessels, is influenced by several factors. The author's previous study demonstrated that selective blockade of nitrogen-monoxide synthase produced a dose-dependent abolition of the regional cerebrovascular CO_2 responsiveness.[32] Co-administration of the L-NAME (NO synthase blocker) and naloxone (general opiate receptor blocker) resulted in almost total abolition of CO_2 responsiveness during hypercapnia.[33] Prostaglandin E_2 of endothelial origin could be a factor in the mediation of the hypercapnia-induced vasodilation in human fetuses.[34] Similarly, both CO_2-induced and Met-enkephalin-induced pial arteriole dilatation involve increased production of dilatory type prostanoids.[35,36]

However, no experimental evidence was found in the literature of the involvement of female sex hormones in the modulation of CO_2 responsiveness of the cerebral vessels.

Ances et al[37] compared CBF responses due to electrical forepaw stimulation before and after brief hypercapnia in male, nonovariectomized female, and ovariectomized female rats. Before hypercapnia the CBF responses were similar for all three groups. Seven minutes after brief hypercapnic exposure, CBF responses to forepaw stimulation were augmented in all groups. However, at both 30 and 60 minutes after hypercapnia, the magnitude of the CBF responses to forepaw stimulation remained elevated in males and ovariectomized females but not in nonovariectomized females. These results suggest that estrogen may modulate the up-regulation of the CBF response observed after transient hypercapnia.

Estrogen is a well-known vasodilator in many local vascular beds. It seems that the effect of estrogen on CBV is the consequence of enhanced cerebral vasodilation. This effect may be mediated in part by prostacyclin, NO, or endothelium-derived hyperpolarizing factor pathways.[2]

CONCLUSIONS

This study demonstrates two different interactions between sex hormones and the regulation of CBV after alteration of Pa_{CO_2}. Lack of female sex hormones seems to result in a significant increase in CBV during hypercapnia. Estrogen and combined hormone treatment seem to prevent the change in CBV after ovariectomy. Conversely, the lack of sex hormones did not modify CBV under hypocapnic conditions. In this study we demonstrated increased CO_2 sensitivity of CBV in the absence of estrogen for the first time. Estrogen treatment prevented this change in CBV.

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REFERENCES

1. Swaab DF, Chung WC, Kruijver FP, Hofman MA, Hestiantoro A. Sex differences in the hypothalamus in the different stages of human life. *Neurobiol Aging* 2003;24(Suppl 1):S1-S16.
2. Krause DN, Duckles SP, Pellegrino DA. Influence of sex steroid hormones on cerebrovascular function. *J Appl Physiol* 2006;101: 1252-1261.
3. Acs N, Szekacs B, Nadasy GL, et al. Effects of ovariectomy and progesterone or combined hormone treatment on small artery biomechanics. *Maturitas* 2000;34:83-92.
4. Acs N, Szekacs B, Nadasy GL, Varbiro S, Kakucs R, Monos E. The effects of ovariectomy and hormone replacement on biomechanical properties of small arteries in rats. *Br J Obstet Gynaecol* 1999;106: 148-154.
5. Sándor P, Coxs van Put J, DeJong W, de Wied D. Continuous measurement of cerebral blood volume in rats with the photoelectric technique: effect of morphine and naloxone. *Life Sci* 1986;39: 1657-1665.
6. Tomita M, Gotoh F, Sato T, et al. Photoelectric method for estimating hemodynamic changes in regional cerebral tissue. *Am J Physiol* 1978; 235:56-63.
7. Pellegrino DA, Galea E. Estrogen and cerebrovascular physiology and pathophysiology. *Jpn J Pharmacol* 2001;86:137-158.
8. Bingham D, Macrae IM, Carswell HV. Detrimental effects of 17-β estradiol after permanent middle cerebral artery occlusion. *J Cereb Blood Flow Metab* 2005;25:414-420.
9. Wise P. Estradiol exerts neuroprotective actions against ischemic brain injury: insights derived from animal models. *Endocrine* 2003;21: 11-15.
10. Orshal JM, Khalil RA. Gender, sex hormones, and vascular tone. *Am J Physiol Integr Comp Physiol* 2004;286:R233-R249.
11. Mersich T, Szelke E, Erdos B, Komjati K, Sandor P. Somatosensory pain does not affect total cerebral blood volume. *Neuroreport* 2007;8: 649-652.
12. Szelke E, Varbiro SZ, Mersich T, et al. Effects of estrogen and progestin on hypothalamic blood flow autoregulation. *J Soc Gynecol Investig* 2005;12:604-609.
13. Welter BH, Hansen EL, Saner KJ, Wei Y, Price TM. Membrane-bound progesterone receptor expression in human aortic endothelial cells. *J Histochem Cytochem* 2003;51:1049-1055.
14. Nakamura Y, Suzuki T, Inoue T, et al. Progesterone receptor subtypes in vascular smooth muscle cells of humane aorta. *Endocr J* 2005;52: 245-252.
15. Lu GP, Cho E, Marx GF, Gibson J. Cerebral hemodynamic response to female sex hormones in the rat. *Microvasc Res* 1996;51:393-395.
16. McCullough LD, Alkayed NJ, Traystman RJ, Williams MJ, Hurn PD. Postischemic hypoperfusion and secondary ischemia after experimental stroke. *Stroke* 2001;32:796-802.
17. McNeill AM, Zhang C, Stanczyk FZ, Duckles SP, Krause DN. Estrogen increases endothelial nitric oxide synthase via estrogen receptors in rat cerebral blood vessels: effect preserved after concurrent treatment with medroxyprogesterone acetate or progesterone. *Stroke* 2002;33:1685-1691.
18. Bain CA, Walters MR, Lees KR, Lumsden MA. The effect of HRT on cerebral hemodynamics and cerebral vasomotor reactivity in postmenopausal women. *Hum Reprod* 2004;19:2411-2414.
19. Sayeed I, Guo Q, Hoffman SW, Stein DG. Allopregnanolone, a progesterone metabolite, is more effective than progesterone in reducing cortical infarct volume after transient middle cerebral artery occlusion. *Ann Emerg Med* 2006;47:381-389.
20. Murphy SJ, Littleton-Kearney MT, Hurn PD. Progesterone administration during reperfusion, but not preischemia alone, reduces injury in ovariectomized rats. *J Cereb Blood Flow Metab* 2002;22: 1181-1188.
21. Littleton-Kearney MT, Klaus JA, Hurn PD. Effects of combined oral conjugated estrogens and medroxyprogesterone acetate on brain infarction size after experimental stroke in rat. *J Cereb Blood Flow Metab* 2005;25:421-426.
22. Murphy SJ, Traystman RJ, Hurn PD, Duckles SP. Progesterone exacerbates striatal stroke injury in progesterone-deficient female animals. *Stroke* 2000;31:1173-1178.
23. Holschneider DP, Scremin OU. Effects of ovariectomy on cerebral blood flow of rats. *Neuroendocrinology* 1998;67:260-268.
24. Resnick SM, Maki PM, Golski S, Kraut MA, Zonderman AB. Effects of estrogen replacement therapy on PET cerebral blood flow and neuropsychological performance. *Horm Behav* 1998;34:171-182.
25. Alkayed NJ, Murphy SJ, Traystman RJ, Hurn PD. Neuroprotective effects of female gonadal steroids in reproductively senescent female rats. *Stroke* 2000;31:161-168.
26. He Z, He YJ, Day AL, Simpkins JW. Proestrus levels of estradiol during transient global cerebral ischemia improve the histological

outcome of the hippocampal CA1 region: perfusion-dependent and -independent mechanism. *J Neurol Sci* 2002;193:79-87.
27. Krejza J, Mariak Z, Nowacka A, Melhem ER, Babikian VL. Influence of 17-β estradiol on cerebrovascular impedance during menstrual cycle in women. *J Neurol Sci* 2004;221:61-67.
28. Bain CA, Lees KR, Lumsden MA, Walters MR. Effect of gonadotropin releasing hormone analog on cerebral hemodynamic in premenopausal women. *Climacteric* 2005;8:193-197.
29. Brackley KJ, Ramsay MM, Broughton PF, Rubin PC. A longitudinal study of maternal blood flow in normal pregnancy and the puerperium: analysis of Doppler waveforms using Laplace transform techniques. *Br J Obstet Gynaecol* 1998;105:68-77.
30. Brackley KJ, Ramsay MM, Pipkin FB, Rubin PC. The effect of the menstrual cycle on human cerebral blood flow: studies using Doppler ultrasound. *Ultrasound Obstet Gynecol* 1999;14:52-57.
31. Wang PN, Liao SQ, Liu RS, et al. Effects of estrogen on cognition, mood and cerebral blood flow in AD: a controlled study. *Neurology* 2000;54:2061-2066.
32. Sandor P, Komjati K, Reivich M, Nyary I. Major role of nitric oxide in the mediation of regional CO_2-responsiveness of the cerebral and spinal cord vessels of the cat. *J Cereb Blood Flow Metab* 1994;14:49-58.
33. Komjati K, Greenberg JH, Reivich M, Sandor P. Interactions between the endothelium-derived relaxing factor/nitric-oxide system and the endogenous opiate system in the modulation of cerebral and spinal vascular CO_2 responsiveness. *J Cereb Blood Flow Metab* 2001;21: 937-944.
34. Kövecs K, Komjati K, Marton T, Skopál J, Sandor P, Nagy Z. Hypercapnia stimulates prostaglandin E_2 but not I_2 release in endothelial cells cultured from microvessels of human fetal brain. *Brain Res Bull* 2001;54:387-390.
35. Armstead WM, Mirro R, Busija DW, Leffler CW. Prostanoids modulate opioid cerebrovascular responses in newborn pigs. *J Pharmacol Exp Ther* 1990;255:1083-1089.
36. Leffler CW, Busija DW. Arachidonic acid metabolites and perinatal cerebral hemodynamics. *Semin Perinatol* 1987;11:32-42.
37. Ances BM, Greenberg JH, Detre JA. Sex differences in the cerebral blood flow response after brief hypercapnia in the rat. *Neurosci Lett* 2001;304:57-60.