Influence of Gender on K⁺-Induced Cerebral Vasodilatation

Sophocles Chrissobolis, BSc(Hons); Christopher G. Sobey, PhD

Background and Purpose—It is not known whether cerebral vasoprotective mechanisms in females include increased function of arterial Kᵢᵣ channels. We hypothesized that vasodilator responses mediated by activation of inwardly rectifying K⁺ (Kᵢᵣ) channels are greater in cerebral arteries of female versus male rats and that this is due to the effects of estrogen.

Methods—Changes in basilar artery diameter were measured with a cranial window preparation in anesthetized Sprague-Dawley rats.

Results—K⁺ (5 and 10 mmol/L) caused greater vasodilatation in females (percent maximum, 21±3% and 58±7%, respectively) versus males (11±1% and 37±4%, respectively; P<0.05). In contrast, vasodilator responses to aprikalim (1 and 3 μmol/L) or acetylcholine (ACh, 1 and 10 μmol/L) did not differ between the genders. The selective Kᵢᵣ channel inhibitor barium ion (30 μmol/L) decreased basilar artery diameter in males but not females (−7±1% versus −2±1%, P<0.05) and selectively inhibited K⁺-induced vasodilatation by ≈50% in both groups. Ovariectomy of female rats resulted in smaller vasodilator effects of K⁺, and chronic treatment of these rats with 17β-estradiol (0.01 mg/kg per day for 7 days) normalized K⁺-induced vasodilatation. Furthermore, the selective M2 muscarinic ACh receptor antagonist methoctramine (1 μmol/L) increased responses to K⁺ in males to levels equivalent to responses in females but had no effect on responses to K⁺ in females.

Conclusions—K⁺ is a more powerful vasodilator in the female versus male cerebral circulation. This difference is estrogen dependent and could be due to a lack of M2 muscarinic ACh receptor–induced inhibition of Kᵢᵣ channel activation by K⁺ in female cerebral arteries. (Stroke. 2004;35:747-752.)

Key Words: basilar artery ■ gender ■ potassium ■ potassium channels

The extracellular concentration of potassium ion (K⁺) in the brain increases from ≈3 mmol/L to between ≈4 and 7 mmol/L during neuronal activity,¹ and in this concentration range, K⁺ elicits marked hyperpolarization and dilatation of cerebral vessels (for review, see elsewhere²). Thus, K⁺-induced cerebral vasodilatation may be an important mechanism for coupling increases in cerebral metabolism and blood flow. K⁺-induced cerebral hyperpolarization and vasodilatation occur primarily via activation of inwardly rectifying K⁺ (Kᵢᵣ) channels.³ Evidence for an involvement of Kᵢᵣ channels in K⁺-induced vasodilatation can be obtained with barium ion (Ba²⁺), which at micromolar concentrations is the most effective and selective Kᵢᵣ channel blocker available.³ Hence, the effect of Ba²⁺ alone on vascular tone reflects basal activity of Kᵢᵣ channels, and because Ba²⁺ constricts cerebral vessels of male animals,⁴,⁵ it is thought that Kᵢᵣ channels normally modulate cerebral arterial tone and help maintain cerebral blood flow.

Compared with men and postmenopausal women, premenopausal women have a lower risk of developing cardiovascular disease, including stroke.⁶,⁷ It is not known whether Kᵢᵣ channel function is influenced by gender. We first tested (1) whether modulation of cerebral arterial tone in vivo by Kᵢᵣ channels differs between genders and (2) whether the magnitude of K⁺-induced cerebral vasodilatation is influenced by gender.

Stimulation of nitrergic nerves in cerebral arteries of males leads to release of nitric oxide (NO) and relaxation of underlying smooth muscle.⁸ This response is enhanced after inhibition of prejunctional M2 muscarinic acetylcholine (ACh) receptors (mAChRs),⁹ suggesting that these receptors modulate neuronal NO release. In an analogous manner, augmented cerebral vasodilator responses to K⁺ occur in male rats during chronic hypertension via depolarization of nitrergic nerves.¹⁰ It is conceivable that depolarization of nitrergic nerves in response to K⁺ may also be modulated by M2 mAChR activity in cerebral arteries of males under normal conditions and that this mechanism is influenced by gender. Having found that responses of the basilar artery to K⁺ are greater in female versus male rats in the first part of this study and that nonselective mAChR blockade by atropine augments responses to 5 mmol/L K⁺ by ≈50% in males (pilot data), we then tested whether effects of K⁺ are normally modulated by M2 mAChRs in males and, to a lesser extent (or not at all), in females.
Materials and Methods
Experiments were performed in Sprague-Dawley rats of either gender (male, n=41; weight, 343±10 g; female, n=35; weight, 277±10 g, mean±SE). The study was approved by the Institutional Animal Experimentation Ethics Committee.

Experimental Protocol
The surgical procedure for animal preparation to measure basilar artery diameter has been described in detail previously.4 Arterial gases were maintained within normal ranges (pH 7.33±0.01; PCO2, 39±1 mm Hg; PO2, 207±7 mm Hg). Cerebrospinal fluid sampled from the cranial window had pH 7.36±0.01, PCO2 of 36±1 mm Hg, and PO2 of 129±1 mm Hg.

Basilar artery diameter was recorded under basal conditions and when stable during application of each drug. In each experiment, control responses to K+ and either ACh (an endothelium-dependent agonist), aprikalim (an opener of ATP-sensitive K+ [KATP] channels), or both were established. Vessel diameter was then allowed to return to control levels between application of vasodilators. The sequence of drug application was randomized. Vessels were then treated for 20 minutes with either Ba2+ (30 μmol/L) or methoctramine (1 μmol/L), an M2 mAChR–selective antagonist. Topical treatment with these pharmacological modulators was continued for the remainder of the experiment, during which responses to K+ and either ACh or aprikalim were retested as appropriate. The effect of the treatment on each vasodilator was determined by comparing the second response with the initial (control) response. In some rats, effects of combined treatments (ie, Ba2+ plus 30 μmol/L Nω-nitro-arginine methyl ester [L-NAME], an inhibitor of NO synthase [NOS], or methoctramine plus L-NAME) were tested. In separate groups of male (n=4) and female (n=5) rats, vasoconstrictor responses to serotonin (0.01 μmol/L) were established, which acted as a control vasoconstrictor for Ba2+. At the conclusion of each experiment, a mixture of 100 μmol/L sodium nitroprusside and 10 μmol/L nimodipine was applied to the vessel to estimate maximum artery diameter.

17β-Estradiol and Vehicle Treatment
A further 12 female rats (215±5 g) were anesthetized (methohexital, 65 mg/kg IP) and given buprenorphine (0.01 mg/kg SC) for analgesia. A bilateral ovariectomy was then performed through a dorsal incision. The skin was sutured closed, and the rat was allowed to recover. After 4 weeks, ovariectomized (OVX) rats had gained ~100 g body weight (318±8 g). Effects on vasodilator responses to K+ of 17β-estradiol treatment (0.01 mg/kg SC per day for 7 days, n=6) were compared with vehicle-treated rats (1% dimethyl sulfoxide SC, n=6). The uterus was harvested and weighed for assessment of the efficacy of 17β-estradiol therapy.

Drugs
All drugs except aprikalim (Rhône Poulenc Rorer) and nimodipine (Calbiochem) were obtained from Sigma Chemical Co. Stock solutions of aprikalim (1 mmol/L) and nimodipine (10 mmol/L) were prepared by dissolving in 50% dimethyl sulfoxide and 50% saline. Subsequent dilutions were made in saline. All other drugs were dissolved and diluted in saline. At the final concentration used, dimethyl sulfoxide alone (≤0.15%) had no effect on basilar artery diameter.

Data Presentation and Statistics
Increases in artery diameter over baseline are expressed as percent of the maximum dilator response achievable by 100 μmol/L sodium nitroprusside plus 10 μmol/L nimodipine. Vasoconstrictor responses to Ba2+ and serotonin are expressed as percent decrease in diameter from baseline. Single comparisons were made by use of Student’s paired or unpaired t test as appropriate. Multiple comparisons were made with an analysis of variance for repeated measures, followed by a Tukey-Kramer test. A value of P<0.05 was considered significant.

Figure 1. Experimental recordings showing time course and magnitude of responses to K+ (a, b) and aprikalim (c, d) in male and female basilar arteries in vivo.
Results

Basilar Artery Diameter In Vivo

Baseline diameter of the basilar artery averaged 234 ± 4 μm (n=41) in males and 251 ± 6 μm (n=23) in females (P<0.05). Maximum diameter was 388 ± 6 μm (n=41) in males and 403 ± 9 μm (n=23) in females (P=0.17). Calculated baseline diameter was 61 ± 1% of maximum diameter in males and 63 ± 1% in females (P=0.13). Arterial pressure averaged 105 ± 3 mm Hg (n=41) in males and 94 ± 4 mm Hg (n=23) in females (P<0.05).

Vasodilator Responses to K⁺, ACh, and Aprikalim

Raising extracellular K⁺ in cerebrospinal fluid from ~3 to 5 and 10 mmol/L caused concentration-dependent vasodilatation of the basilar artery in male and female rats (Figures 1a, 1b, and 2a). Responses to these concentrations of K⁺ were 100% and 56%, respectively, greater in females than males (P<0.05). In contrast, responses to aprikalim (Figures 1c, 1d, and 2b) and ACh (Figure 2c) were each similar between the genders.

Effects of Ba²⁺

Ba²⁺ (30 μmol/L) caused ~7% vasoconstriction in males (Figure 3a) but had no significant effect on artery diameter in females (Figure 3a). In contrast, vasoconstrictor responses to serotonin (0.01 μmol/L) were similar in magnitude in males and females (Figure 3b). Ba²⁺ inhibited vasodilator responses to K⁺ in both males and females by 30% to 55% (Figures 4a and 4c) but had no effect on vasodilator responses to aprikalim (Figures 4b and 4d).

After inhibition of responses to K⁺ by Ba²⁺, some female rats were treated further with a combination of Ba²⁺ plus l-NAME (30 μmol/L). The purpose of these experiments was to test whether NOS activity contributes to the larger responses to K⁺ in females. Relative to the original baseline level, l-NAME further constricted the basilar artery to ~17% ± 5% (n=6) but had no additional inhibitory effect on responses to 5 and 10 mmol/L K⁺ (control, 18 ± 5% and 56 ± 13%, respectively; Ba²⁺ treated, 9 ± 2% and 43 ± 12%; Ba²⁺ plus l-NAME treated, 7 ± 1% and 40 ± 9%; n=7). Thus, NOS is apparently not involved in any Ba²⁺-resistant component of K⁺-induced vasodilatation in females, as found previously in male Sprague-Dawley rats. However, in the presence of Ba²⁺ and l-NAME, responses to 1 and 10 μmol/L ACh were markedly inhibited (control, 15 ± 4% and 32 ± 8%; Ba²⁺ plus l-NAME treated, 3 ± 2% and 10 ± 4%; P<0.05; n=5), confirming that l-NAME treatment inhibited NO production.

Effect of Ovariectomy and Estradiol Treatment

OVX female rats treated with vehicle for 7 days gained ~20 g body weight (316 ± 16 to 336 ± 6 g, n=6), whereas weights
of OVX rats treated with 17β-estradiol were virtually unchanged (320±5 to 319±7 g, n=6). Uterine weight was increased by 17β-estradiol treatment (362±31 versus 164±17 mg in vehicle-treated OVX rats, P<0.05). Arterial pressure did not differ between the 2 treatment groups (72±8 and 76±5 mm Hg in vehicle- and 17β-estradiol–treated groups, respectively). Baseline diameter of the basilar artery averaged 236±10 μm in vehicle-treated rats and 280±17 μm in rats treated with 17β-estradiol (P<0.05). Maximum artery diameter and calculated baseline diameter were 396±14 μm and 60±3%, respectively, in vehicle-treated rats and 402±7 μm and 70±5% in 17β-estradiol–treated rats (both P>0.05).

Similar to the comparison of males versus females (Figure 2a), vasodilator responses to 5 and 10 mmol/L K+ were 120% (P<0.05) and 55% (P=0.13) greater, respectively, in 17β-estradiol–treated than vehicle-treated rats (Figure 5). However, there was no difference in the effect of 30 μmol/L Ba2+ on diameter of OVX rats treated with vehicle or 17β-estradiol (−3.1±0.6% versus −3.8±0.9%, respectively).

Effects of Methoctramine

**Males**

Application of methoctramine (1 μmol/L) had no effect on basilar artery diameter (see Figure 6 legend), but it augmented responses to K+ by 40% to 65% (Figure 6a). Methoctramine had no effect on responses to ACh (control responses for 1 and 10 μmol/L ACh, 20±4% and 51±6%, respectively; methoctramine treated, 19±4% and 45±7%; n=8) or aprikalim (control responses for 1 and 3 μmol/L aprikalim, 11±6% and 44±15%, respectively; methoctramine treated, 16±4% and 51±14%; n=5).

**Females**

Methoctramine had no effect on basilar artery diameter (see Figure 6 legend). In contrast to males, methoctramine had no effect on K+-induced vasodilation in females (Figure 6b). In the presence of methoctramine, some male rats were further treated with a combination of methoctramine and L-NAME, and effects on K+ and ACh were retested (baseline diameters: control, 230±6 μm; methoctramine treated, 236±5 μm; methoctramine plus L-NAME treated, 215±7 μm; P<0.05 versus control plus methoctramine treated). However, augmented responses to K+ in the presence of

![Figure 4](http://stroke.ahajournals.org/)

**Figure 4.** a, b, Effect of 30 μmol/L Ba2+ on vasodilator responses to K+ (a, n=7) and aprikalim (b, n=4) in male rats. c, d, Effect of Ba2+ on vasodilator responses to K+ (c, n=13) and aprikalim (d, n=3) in female rats. Baseline diameters are as follows: control, 242±11 μm; Ba2+ treated, 238±17 μm (a); control, 244±12 μm; Ba2+ treated, 252±11 μm (b); control, 248±7 μm; Ba2+ treated, 239±7 μm (c); and control, 240±3 μm; Ba2+ treated, 238±2 μm (d). *P<0.05 vs control.

![Figure 5](http://stroke.ahajournals.org/)

**Figure 5.** Vasodilator responses to K+ in vehicle-treated (n=6) and 17β-estradiol (17β-E2)–treated (n=6) OVX rats. Baseline diameters are as follows: vehicle treated, 235±12 μm; 17β-estradiol treated, 279±18 μm. *P<0.05 vs vehicle-treated rats.
Ba\(^{2+}\)), indicating that this response is entirely mediated by K\(_{ir}\) channels. However, in the present and previous studies\(^4,10,12\) we found that topical application of Ba\(^{2+}\) incompletely inhibits dilator responses of the basilar artery to K\(^{+}\) in vivo. Because this Ba\(^{2+}\)-resistant component of the response to K\(^{+}\) in vivo is also insensitive to inhibitors of K\(_{ATP}\), voltage-dependent or large-conductance calcium-activated K\(^{+}\) channels, NOS, or Na-K\(^{+}\)-ATPase, we speculate that the entire response is indeed mediated by K\(_{ir}\) channels but that Ba\(^{2+}\) cannot fully access and block K\(_{ir}\) channels when applied topically in this manner.

The greater magnitude of vasodilator responses was selective for K\(^{+}\) in females and was reduced by ovariectomy and restored by 17\(\beta\)-estradiol therapy. Enhanced vasorelaxation to ACh and greater levels of NO release have been reported in several vessels from females compared with males.\(^13,15\) However, similar responses to ACh in either gender in the present study perhaps suggest that there is no gender difference in endothelium-dependent vasodilatation in the rat basilar artery, as has been reported in a study of rabbit thoracic aorta, despite greater basal NO release.\(^16\) Our data also suggest that there is no gender difference in cerebral vascular responses mediated by K\(_{ATP}\) channels.

Our data are consistent with those of Knot and colleagues,\(^11\) who similarly found K\(^{+}\)-induced hyperpolarization and relaxation of the female isolated posterior cerebral artery to be Ba\(^{2+}\) sensitive. Interestingly, in contrast to its constrictor effect in males, we found that Ba\(^{2+}\) had no effect on basilar artery diameter in females, suggesting little or no role for K\(_{ir}\) channels in modulating basal artery tone in females. Knot et al likewise reported little or no evidence for a contribution of K\(_{ir}\) channels to resting membrane potential of female cerebral arteries.\(^11\) Thus, it is possible that greater responses to K\(_{ir}\) channel activation in females are related to a higher number of inactive, but activatable, K\(_{ir}\) channels representing a larger “reserve” available for responding to a K\(_{ir}\) channel opener such as K\(^{+}\). The molecular mechanisms underlying such a gender difference in K\(_{ir}\) channel activation appear to be due to effects of estrogen because 17\(\beta\)-estradiol treatment in O VX females normalized responses to K\(^{+}\). Another study\(^17\) reported no difference in relaxant responses to 15 mM/L K\(^{+}\) in the isolated middle cerebral artery of males versus females. In contrast, we have found that responses of the basilar artery in vivo to 15 mM/L K\(^{+}\) are greater in females than males (as presented here for 5 and 10 mM/L K\(^{+}\); data not shown). This difference could be related to the differences in experimental models or perhaps to the different basal concentrations of K\(^{+}\) used (3 versus 6 mM/L).

We also tested whether NO might be involved in partly mediating the greater responses to K\(^{+}\) in females, given evidence for increased bioactivity of NO in females\(^7\) and that neuronal NO mediates augmented cerebral vasodilator responses to K\(^{+}\) during chronic hypertension.\(^10\) However, we found no effect of \(\text{L-NAME}\) on responses to K\(^{+}\) in females, suggesting no role for NOS in this response, as has been reported in males.\(^4\)

Role of Muscarinic Receptor Activity
M2 mACHRs are expressed in the rat basilar artery.\(^18\) We found that methoctramine, a selective M2 mACHR antag-
nist, augmented K⁺-induced dilatation of the basilar artery in males. This finding is consistent with an inhibitory effect of M2 mACHR activity on Kir-mediated responses to K⁺ in male rats. In contrast, methoctramine had no effect on K⁺-induced vasodilatation in females. Thus, we speculate that a lack of M2 mACHR-mediated inhibition of K⁺-induced vasodilatation in females may at least partly explain why responses to K⁺ are greater in females versus males. This modulatory effect of M2 mACHR activity in males does not appear to involve K⁺-induced release of NO from nitric nerves, however, because t-NNAME did not attenuate the augmented responses to K⁺ during methoctramine treatment. Methoctramine also had no effect on vasodilatation by the K ATP channel opener aprikalim. Because it is well established that K ATP channels are regulated by cAMP-dependent protein kinase, it is unlikely that activity of this enzyme is enhanced by methoctramine. The lack of effect of methoctramine on baseline diameter also suggests that M2 mACHR activity does not contribute substantially to cerebral vascular tone under basal conditions.

Although the source of endogenous ACh, which appears to modulate responses to K⁺ in males, is presumably cholinergic neurons innervating the basilar artery, the location of its target M2 mACHRs is currently unclear. In addition to neurons, M2 mACHRs are expressed on both endothelial and vascular smooth muscle cells. It seems unlikely that endothelial M2 mACHRs are involved in such an action, however, because endothelial denudation has no effect on K⁺-induced cerebral vasorelaxation in male rats. Perhaps decreased cAMP levels, which result after M2 mACHR activation in gastric smooth muscle cells, lead to decreased vascular smooth muscle Kir2.1 channel activity because cAMP enhances Kir2.1 current in cultured cells. Such a mechanism is plausible in the rat basilar artery in which mRNA for both M2 mACHR and Kir2.1 channels is expressed.

We are alert to the limitations of using pharmacological antagonists to determine the functional role of mACHR subtypes because these agents typically display an overlapping pharmacological profile with respect to actions on the 5 main mACHR subtypes, making interpretations of results from their use potentially difficult. Nevertheless, methoctramine is currently regarded as the best available agent for selectively inhibiting M2 mACHRs and is widely used for this purpose. Future studies using M2 mACHR knockout mice would help to ascertain more directly the vascular effects of M2 mACHRs on K⁺/Kir-mediated cerebral vasodilator responses.

The results of the present study demonstrate that K⁺ is a more powerful vasodilator in the cerebral circulation of female versus male rats. This difference appears to be dependent on effects of estrogen and could involve a lack of M2 mACHR-mediated modulation of Kir channel activation in response to K⁺ in females.

Acknowledgments

Dr Sobey is supported by an NHMRC R.D. Wright Career Development Award (209160). S. Chrissobolis is supported by an Australian Postgraduate Award. We are grateful to Mirna Boujaoude for her assistance in performing ovariectomy surgeries and daily injections of rats and to Dr Frank Faraci for helpful comments during preparation of this manuscript.

References

Influence of Gender on K⁺-Induced Cerebral Vasodilatation
Sophocles Chrissobolis and Christopher G. Sobey

Stroke. 2004;35:747-752; originally published online February 5, 2004;
doi: 10.1161/01.STR.0000116867.28589.3A
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/35/3/747

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office.
Once the online version of the published article for which permission is being requested is located, click
Request Permissions in the middle column of the Web page under Services. Further information about this
process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/