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Endothelium-Dependent Gender Differences in the Response of the Rat Pulmonary Artery to the Thromboxane Mimic (U46619)[1]

CYNTHIA M. CUNARD, YVONNE T. MADDOX and PETER W. RAMWELL
Department of Physiology and Biophysics, Georgetown University Medical Center, Washington, District of Columbia
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ABSTRACT

The pulmonary arteries of rats were studied in order to determine the existence of sexual dimorphism. Gender differences, in the sensitivity (EC_{50}) and maximum contractility (T_{max}) of ring preparations of the main pulmonary arteries of adult male and female rats, were evaluated with the synthetic endoperoxide analog [(15*S*)-hydroxy-11α,9α-(epoxymethano)-prosta-5Z, 13E-dienoic acid,] (U46619) and norepinephrine. There were no significant gender differences in the T_{max} values obtained with either U46619 or norepinephrine. However, when the intimal surface of vessel segments from female rats was rubbed, U46619 but not norepinephrine elicited a significantly lower T_{max}. In contrast, no change in T_{max} was observed with denuded vessel segments from males. Removal of the endothelium did not significantly affect the EC_{50} of U46619 or norepinephrine in segments from either sex. The inhibitory effect of verapamil on the U46619-induced contractile response was studied on both intact and denuded vessels from rats of both gender. The T_{max} of intact vessels from males but not females was significantly attenuated by verapamil ($P < .05$). The EC_{50} values with verapamil were not significantly different in any of the vessel preparations. We suggest that the endothelium of the pulmonary artery of female rats significantly potentiates the contractile response to U46619 and attenuates the inhibitory effect of verapamil.

Sexual dimorphism in the vascular reactivity of isolated pulmonary blood vessels is of considerable interest owing to the reported gender differences in the incidence of primary pulmonary hypertension. After sexual maturation, women present with this disease three times more frequently than men (Wagenvoort and Wagenvoort, 1970).

Recent studies demonstrate that the endothelium mediates the response of the underlying smooth muscle to several agonists (DeMey and Vanhoutte, 1982; Singer and Peach, 1983) and may be involved in the development of pulmonary hypertension (Molteni *et al.*, 1984). An EDRF was reported by Furchgott and Zawadski (1980) and has subsequently been verified in various vascular preparations including the human pulmonary artery (Chand and Altura, 1981; Maddox *et al.*, 1985).

In addition to EDRF, the pulmonary endothelium may also release a contractile substance. Exuded lung surface fluid and lymph fluid collected during vasoconstriction, caused by bilateral and unilateral hypoxia, elicited contractile responses of isolated helical strip preparations of the canine pulmonary artery (Benumof *et al.*, 1978). In addition, hypoxic vasoconstriction of porcine pulmonary artery rings requires the presence of the endothelium and may involve the release of an endothelium-dependent contractile factor (Holden and McCall, 1983). Furthermore, the supernates obtained from cultured bovine aortic and pulmonary endothelial cells contract preparations of the bovine pulmonary artery (O'Brien and McMurty, 1983).

The purpose of this study was to investigate in pulmonary arteries the role of the endothelium in mediating vascular responses to the endoperoxide analog which mimics thromboxane A_2, U46619 (Coleman *et al.*, 1981). The stable thromboxane mimic U46619 was chosen because of reports that primary pulmonary hypertension was associated with platelet deposition in the pulmonary circulation (Wagenvoort and Wagenvoort, 1970) and due to the unstable nature of authentic thromboxane A_2. In addition, the effect of the calcium entry blocker, verapamil, in modifying the vascular responses of these blood vessels to U46619 was evaluated. Verapamil was chosen because this drug is currently used in the clinic for treating primary pulmonary hypertension (Malcic and Richter, 1985). We found that there were prominent gender differences which were revealed when the vessels were de-endothelialized.

Methods

Animals. A total of 51 mature (12 weeks of age) Sprague-Dawley rats from the Charles River Breeding Laboratories (Wilmington, MA) were housed in a controlled environment with food and water *ad libitum*. The females and males weighed 234.2 ± 6.42 and 260.83 ±

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ABBREVIATIONS: EDRF, endothelium-dependent relaxing factor; KRB, Krebs-Ringer bicarbonate; PG, prostaglandin.

11.80 g, respectively, at the time of these studies. Within each sex, animals were randomly placed into two groups. Vascular preparations obtained from animals in one group had the endothelium mechanically removed and vessel preparations obtained from animals in the second group did not. Animals were anesthetized before surgery with sodium pentobarbital (50 mg/kg i.p.; Abbott Laboratories, N. Chicago, IL).

Vessel preparation. Main pulmonary artery rings (2–2.5 mm in length) proximal to the lung were excised from male and female rats. The vessel segments were placed immediately in cold KRB solution equilibrated with 5% CO_2 in oxygen and consisting of (millimolar): NaCl, 119.2; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHPO_4$, 1.2, $NaHCO_3$, 26.2; and glucose, 5.6. Segments were then trimmed of fat and connective tissue. Care was taken to avoid stripping the endothelium.

Endothelial removal. The intimal surface of the rat main pulmonary artery ring preparation was gently rubbed using a watchmaker's forcep, according to the method of DeMey and Vanhoutte (1982).

Vascular reactivity. Pulmonary artery rings were suspended in tissue baths (3 ml) containing KRB at 37°C, aerated with 5% CO_2 in oxygen. Each preparation was equilibrated for 1 hr with 1 g of tension and a KRB perifusion rate of 2.6 ml/minute. After the equilibration period, cumulative dose-response curves were generated using the agonist U46619 in a dose range of 10^{-10} to 10^{-7} M. In preliminary studies to ascertain reproducibility, three consecutive cumulative dose-response curves were generated to U46619 (fig. 1). There were no significant differences between any of the three curves. Vessel rings were washed for 1.5 hr with KRB at 2.6 ml/min before a second curve was generated. In studies with verapamil, a second cumulative dose-response curve to U46619 was generated after the preparations were incubated in the tissue bath for 30 min in the presence of verapamil (1.36×10^{-6} M). The wash procedure was repeated, the vessel rings were incubated with a second dose of verapamil (4.08×10^{-6} M) and cumulative dose-response curves to U46619 were obtained as described previously. Cumulative dose-response curves were generated for norepinephrine (10^{-10} to 10^{-6} M) in intact and denuded rat pulmonary artery rings from both sexes, as described above. Changes in the tension generated by the vessel rings was measured isometrically using force-displacement transducers (Harvard No. 363), prerecording modules (Harvard No. 350) and a chart recorder (Harvard No. 486).

Drugs. The synthetic endoperoxide analog which mimics thromboxane, U46619 [(15*S*)-hydroxy-11α,9α-(epoxymethano)-prosta-5Z, 13E-dienoic acid] was prepared by evaporating 20 μl of a stock solution (1 mg/ml in ethanol) to dryness under nitrogen and redissolving it in 0.9% saline. The stock of U46619 (Upjohn Co., Kalamazoo, MI) was a gift of Dr. John Pike. Verapamil HCl (Knoll Pharmaceuticals, Whippany, NJ) was prepared daily by adding 2 mg of verapamil HCl to 10 ml of 0.9% saline. Norepinephrine (L-Arterenol HCl) was supplied by Sigma Chemical Co. (St. Louis, MO). The norepinephrine solutions were prepared in 0.9% saline and stabilized with ascorbic acid (10^{-4} M). The prepared drugs were kept at 4°C and added to the bath in volumes less than 100 μl.

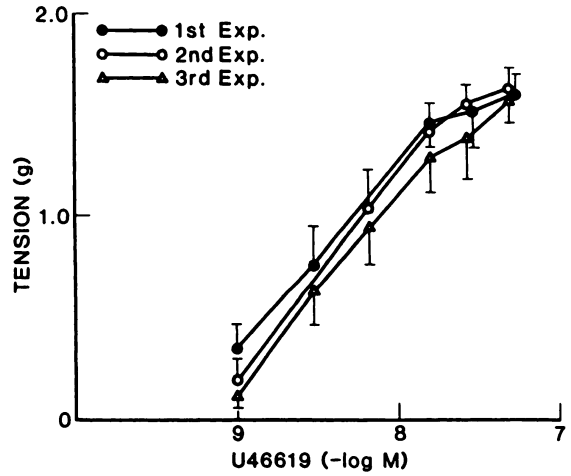

Fig. 1. Three consecutive control cumulative dose-response curves to U46619 in intact segments of the rat pulmonary artery dissected from males and females and denuded male pulmonary preparations in the absence of the verapamil diluent, saline [Experiment (Exp.) 1; ●] and the presence of 20 μl (Exp. 2; ○) and 40 μl (Exp. 3; △) of saline. Each point on the curve represents at least five observations.

Statistical analyses. The maximum contractile responses to U46619 were statistically compared using the Newman-Keuls multiple range test. The EC_{50} values were determined from linear regression analysis obtained from the cumulative dose-response curves. The EC_{50} values were compared using the Newman-Keuls multiple range test. The contractile responses to norepinephrine were statistically compared between groups using the Student's *t* test for unpaired data. The level of significance was defined as $P < .05$.

Results

The EC_{50} and T_{max} values obtained with U46619 in rat pulmonary artery rings from male and female rats are shown in table 1. For convenience we report the T_{max} and EC_{50} separately and begin with the data obtained from intact vessels of both genders followed by the values obtained in denuded vessels.

The T_{max} elicited by U46619 in intact pulmonary artery segments from male and female rats was not significantly different. The T_{max} of denuded vessels from female rats was significantly lower ($P < .05$) than the response of intact segments. In contrast there was no significant difference in T_{max} between intact and denuded segments from male vessels.

Verapamil (4.08×10^{-6} M) significantly ($P < .05$) reduced the T_{max} of intact segments of pulmonary artery from male rats. In conrtrast verapamil did not significantly reduce the T_{max} of intact or denuded ring segments from female rats. The EC_{50} values (10^{-8} M) for U46619 obtained in ring segments from male and female vessels were not significantly affected by either removal of the endothelium or exposure to verapamil.

The cumulative dose-response curves elicited by norepinephrine (10^{-9} to 10^{-6} M) in intact and denuded vessel rings dissected from male and female rat pulmonary arteries are shown in figure 2. There were no significant gender differences in T_{max} elicited by norepinephrine using intact vessels. Furthermore, removal of the endothelium did not significantly affect the contractile responses or the sensitivities to norepinephrine in pulmonary arterial segments from males or females.

The T_{max} values obtained with norepinephrine in vessels from males, intact (2.3 ± 0.3 g) or denuded (1.75 ± 0.4 g), were not significantly different from each other or from those obtained with U46619 which were 1.86 ± 0.1 and 1.52 ± 0.1 g for intact and denuded, respectively (table 1; fig. 2). The T_{max} values obtained with norepinephrine in intact and denuded segments from the female were 2.39 ± 0.3 and 2.2 ± 0.3 g, respectively. These values were not significantly different from each other or from the U46619-induced T_{max} in intact rings. However, the norepinephrine-induced T_{max} in denuded segments from females was significantly higher ($P < .001$) than the U46619-induced T_{max} in denuded pulmonary rings from females which was 0.81 ± 0.2 g.

Discussion

The role of the endothelium in the contractile response of isolated pulmonary artery preparations of the rat was studied

TABLE 1

Reactivity of endothelium-intact and denuded rat pulmonary artery preparations from both sexes to U46619

All statistical analyses were performed using the Newman-Keuls Multiple Range test. Numbers in parentheses, number of animals evaluated.

Treatment	EC_{50}[h]			Maximum Contractility (T_{max})[i]		
	Male	Female	P	Male	Female	P
Intact	2.29 ± 0.81 (7)	3.02 ± 0.91 (7)	NS	1.86 ± 0.11 (8)	1.86 ± 0.16 (8)	NS
Verapamil 1.36×10^{-6} M	3.31 ± 0.87 (8)	2.57 ± 0.83 (8)	NS	1.41 ± 0.17 (8)	1.67 ± 0.21 (8)	NS
Verapamil 4.08×10^{-6} M	4.07 ± 0.91 (8)	3.09 ± 0.89 (8)	NS	0.86 ± 0.14[b,d] (8)	1.43 ± 0.23 (8)	NS
Denuded	1.99 ± 0.71 (8)	4.26 ± 0.85 (6)	NS	1.52 ± 0.13 (8)	0.81 ± 0.24[b,d] (8)	NS
Verapamil 1.36×10^{-6} M	4.26 ± 0.89 (7)	3.98 ± 0.91 (4)	NS	1.02 ± 0.24 (8)	0.67 ± 0.27[a,c,f] (8)	NS
Verapamil 4.08×10^{-6} M	3.55 ± 0.83 (6)	4.68 ± 0.93 (4)	NS	0.75 ± 0.25[b,c,f] (8)	0.53 ± 0.25[a,c,e,g] (8)	NS

[a] $P < .01$ compared to intact male; [b] $P < .05$ compared to intact male; [c] $P < .01$ compared to intact female; [d] $P < .05$ compared to intact female; [e] $P < .01$ compared to intact female, verapamil 1.36×10^{-6} M; [f] $P < .05$ compared to intact female, verapamil 1.36×10^{-6} M; [g] $P < .05$ compared to denuded male; [h] Log dose U46619 (10^{-6} M) yielding 50% maximum response; [i] maximum tension (grams).

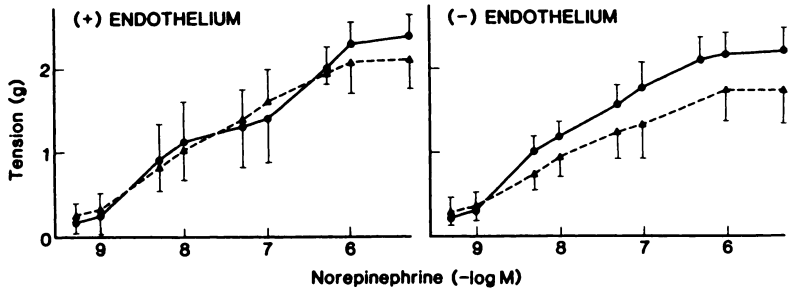

Fig. 2. Cumulative dose-response curves to norepinephrine in intact [(+)-Endothelium] and denuded [(−)-Endothelium] rat pulmonary artery segments dissected from females (●) and males (▲). Each point on the curve represents at least four observations.

by contracting the tissue with the thromboxane mimic U46619 and norepinephrine before and after the lumen was rubbed. The role of the endothelium was studied further by comparing preparations from sexually mature male and female rats. No gender differences in either sensitivity or contractility to U46619 or norepinephrine was apparent. However, when the endothelium was removed, a prominent effect was observed with the vessel from the female but not from the male. The denuded vessel from the female exhibited significantly less tension in response to U46619 than when the vessel was intact. This decrease in contractility of the vessel from the female was seen with the thromboxane mimic U46619 and not norepinephrine. Consequently, it is unlikely that the decrease in contractility can be related to smooth muscle damage; because if this were the case, one would expect the response to both agonists to be compromised. Moreover one might also expect a decrease in sensitivity if damage had been done and this was not the case. There was no significant change in the sensitivity of the vessels of the male or female rats stimulated with either U46619 or norepinehrine before or after denuding.

A possible explanation for this gender difference after removal of the endothelium may relate to the presence of a contractile factor in the endothelium which can be expressed by U46619 but not by norepinephrine. In fact a contractile peptide released from cultured bovine aortic endothelial cells has been described. Hickey *et al.* (1985) describe an endothelium-derived peptide which contracts in a dose-dependent manner bovine, porcine and canine left anterior descending coronary arteries that have been denuded. O'Brien *et al.* (1985) have demonstrated an endothelium-derived factor which produced vasoconstriction in isolated rat kidneys and Langendorff-perfused rabbit hearts, yet had no effect on isolated rat lungs. In addition bovine pulmonary endothelial cells also release a peptide which constricts the bovine pulmonary artery (O'Brien and McMurty, 1983). Thus, it is possible that such a vasoactive peptide exists in the endothelium of the pulmonary artery of the rat and is expressed by U46619 and not norepinephrine. Furthermore the expression is likely more prominent in the endothelium of the female.

The gender difference in the response to U46619 after removal of the endothelium may speak to the possibility that there is a gender difference in receptors to the thromboxane. We have described such gender differences previously using spiral preparations of the rat aorta in which the gender differentiated response was elicited by U46619 and other cyclooxygenase products but not by norepinephrine (Karanian *et al.*, 1981a,b). Aortic strips from male rats were more contractile to these eicosanoids than strips from females. We concluded from these studies that there was a sexual dimorphism in prostaglandin receptors. The smooth muscle of the pulmonary arteries of female rats may also have a decreased number of specific thromboxane receptors as compared to males.

The gender differences observed with the calcium channel blocker verapamil are of considerable interest in that again the data relates to a gender difference in the endothelium. Verapamil attenuated the response of the vessel from the male to U46619 more than the vessel of the female. These data are consonant with the idea that the expression of the putative contractile factor elicited by U46619 from the endothelium of vessels from females is not blocked by verapamil. Support for this suggestion can also be derived from a recent abstract by Miller and Stoclet (1984) who tested the effect of another

calcium blocker flunarizine, on another product of the cyclooxygenase pathway namely $PGF_{2\alpha}$. They found flunarizine was more effective in blocking the contractile response to high dose $PGF_{2\alpha}$ in the denuded rat aorta preparation derived from the female rat than when the vessel was intact. However, at submaximal doses of $PGF_{2\alpha}$ this phenomenon was reversed and the female intact preparations were less sensitive to the inhibitory actions of flunarazine than the corresponding denuded vessels. The disparity of these findings may relate to regional differences between the aortic and pulmonary endothelium of the female rat, the different agonist used or the different calcium entry blocker that was evaluated. However, both studies suggest that the female endothelium may mediate the sensitivity of the rat aorta and rat pulmonary artery to the inhibitory actions of flunarazine and verapamil, respectively. Moreover, the present study suggests that the sensitivity of the rat pulmonary artery to the inhibitory actions of verapamil is not only endothelial mediated but gender differentiated.

In conclusion, data are presented which demonstrate the existence of a sexual dimorphism in the degree of endothelium potentiation of U46619-induced contractility in rat pulmonary arterial segments. These findings imply that the pulmonary endothelium of the female rat may enhance the contractile response to the thromboxane mimic through the release of a contractile substance.

One important feature of this study is the finding that the male intact rat pulmonary artery is more sensitive to the inhibitory actions of verapamil than corresponding female preparations. Other investigators have reported that there are "responders" and "nonresponders" to pulmonary vasodilator drugs (Rich *et al.*, 1983). We suggest that this may be related to the presence of a contractile factor that is expressed or released to a greater degree in these nonresponders. This finding may have clinical relevance as the standard doses of pulmonary vasodilators used in the treatment of pulmonary hypertension in this group of nonresponding patients may be ineffective.

References

BENUMOF, J. L., MATHERS, J. M. AND WAHRENBROCK, A.: The pulmonary interstitial compartment and the mediator of hypoxic pulmonary vasoconstriction. Microvasc. Res. **15:** 69–75, 1978.

CHAND, N. AND ALTURA, B. M.: Acetylcholine and bradykinin relax intrapulmonary arteries by acting on endothelial cells: Role in lung vascular diseases. Science (Wash. DC) **213:** 1376–1379, 1981.

COLEMAN, R. A., HUMPHREY, P. P. A., KENNEDY, I., LEVY, G. P. AND LUMLEY, P.: Comparison of the actions of U-46619, a prostaglandin H_2-analogue, with those of prostaglandin H_2 and thromboxane A_2 on some isolated smooth muscle preparations. Br. J. Pharmacol. **73:** 773–778, 1981.

DEMEY, J. G. AND VANHOUTTE, P. M.: Heterogeneous behavior of the canine arterial and venous wall. Importance of the endothelium. Circ. Res. **51:** 439–447, 1982.

FURCHGOTT, R. F. AND ZAWADSKI, J. V.: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature (Lond.) **288:** 373–376, 1980.

HICKEY, K. A., RUBANYI, G., PAUL, R. J. AND HIGHSMITH, R. F.: Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. Am. J. Physiol. **248:** C550–C556, 1985.

HOLDEN, W. E. AND MCCALL, E.: Hypoxic vasoconstriction of porcine pulmonary artery strips *in vitro* requires an intact endothelium. Am. Rev. Respir. Dis. **127:** A301, 1983.

KARANIAN, J. W., MORAN, F. M., RAMEY, E. R. AND RAMWELL, P. W.: Gender differences in prostaglandin receptors of rat aorta. Br. J. Pharmacol. **72:** 10–12, 1981a.

KARANIAN, J. W., RAMEY, E. R. AND RAMWELL, P. W.: Effect of gender on development and diurnal rhythm of prostaglandin receptors or rat aorta. Br. J. Pharmacol. **73:** 903–907, 1981b.

MADDOX, Y. T., CUNARD C. M., SHAPIRO, R., KAWAGUCHI, A., GOLDMAN, M., LOWER R. F. AND RAMWELL, P. W.: A comparison of the contractile response of rodent and human pulmonary vascular segments to eicosanoids. *In* Prostaglandins, Leukotrienes and Lipoxins, ed. by M. Bailey, pp. 267–273, Plenum Press, New York, 1985.

MALCIC, I. AND RICHTER, D.: Verapamil in primary pulmonary hypertension. Br. Heart J. **53:** 345–347, 1985.

MILLER, R. C. AND STOCLET, J.: Effect of Endothelium on Agonist Sensitivity to Flunarazine in Rat Isolated Aorta, International Symposium on Calcium Entry Blockers and Tissue Protection, Rome, p. 26, 1984.

MOLTENI, A., WARD, W. E., TS'AO, C., PORT, C. D. AND SOLLIDAY, N. H.: Monocrotaline-induced pulmonary endothelial dysfunction in rats. Proc. Exp. Biol. Med. **176:** 88–94, 1984.

O'BRIEN, R. F. AND MCMURTY, I. F.: Endothelial cell (EC) supernates contract bovine pulmonary artery (PA) rings. Am. Rev. Respir Dis. **127:** A337, 1983.

O'BRIEN, R. F., OWASOYO, J., MCMURTY, I. AND GILLESPIE, M.: Sustained coronary vasoconstriction in isolated hearts caused by endothelial cell conditioned media. Fed. Proc. **44:** A6249, 1985.

RICH, S., MARTINEZ, I., LAM, W., LEVY, P. AND ROSEN, D.: Reassessment of the effects of vasodilator drugs in primary hypertension: Guidelines for determining a pulmonary vasodilator response. Am. Heart J. **105:** 119–127, 1983.

SINGER, H. A. AND PEACH, M. J.: Endothelium-dependent relaxation of rabbit aorta. I. Relaxation stimulated by arachidonic acid (AA). J. Pharmacol. Exp. Ther. **227:** 790–795, 1983.

WAGENVOORT, C. A. AND WAGENVOORT, N.: Primary pulmonary hypertension. A pathologic study of the lung vessels in 156 clinically diagnosed cases. Circulation **62:** 1163–1184, 1970.

Send reprint requests to: Cynthia M. Cunard, Department of Physiology and Biophysics, Georgetown University Medical Center, 3900 Reservoir Rd. N.W., Washington, DC 20007.