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# Postjunctional $\alpha_2$-adrenoceptors in the rat tail artery: effect of sex and castration

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**Abstract**

To investigate sex-related differences in vasoconstrictor responses to postjunctional $\alpha_2$-adrenoceptor activation, isolated ring segments of tail arteries from Fischer-344 rats were studied. Addition of the $\alpha_2$-adrenoceptor agonist, UK-14304 [5-bromo-6-(2-imidazoline-2yl)-aminol-quinoxaline], enhanced vasoconstriction to the selective $\alpha_1$-adrenoceptor agonist, methoxamine, in arteries from both males and females. The response to UK-14304 was significantly greater in arteries from males as compared to female arteries. Addition of $\alpha_2$-adrenoceptor antagonist, idazoxan or rauwolscine, shifted norepinephrine concentration response curves to the right. Antagonist effects also tended to be greater in arteries from males as compared to females. After gonadectomy, male–female differences persisted; thus, removal of sex hormones in either males or females did not alter responses to either agonists or antagonists of $\alpha_2$-adrenoceptors. These findings suggest that sex differences in $\alpha_2$-adrenoceptor function are not maintained by either male or female gonadal steroid hormones but may be developmentally regulated. 

*Keywords:* Sex; $\alpha_2$-Adrenoceptor; Gonadal steroid; Rauwolscine; Idazoxan; UK-14304

## 1. Introduction

Sex-related differences in cardiovascular response and risks for cardiovascular disease have been well-established (Beale and Collins, 1996). There is a sex-related dichotomy in the incidence of cardiovascular disorders such as coronary heart disease and angina as well as other circulatory disorders, such as stroke, hypertension and Raynaud's disease (White et al., 1995). Moreover, male–female differences in vascular reactivity have been observed both in vivo and in vitro (White et al., 1997). Although many sex-related differences have been characterized, the functional basis for differences in cardiovascular reactivity and the possible role of sex hormones remain ill-defined.

It has been reported that responses of humans to the $\alpha_2$-adrenoceptor agonist, clonidine, differ with sex (Del Rio et al., 1993), although cardiovascular effects of clonidine in women were not affected by estrogen therapy (Del Rio et al., 1997). Function of prejunctional $\alpha_2$-adrenoceptors to inhibit norepinephrine release has been reported to differ with sex in rats (Du et al., 1991), and $\alpha_2$-adrenoceptor-mediated inhibition of norepinephrine release in the rat hypothalamus has been shown to be attenuated by estradiol treatment (Karkanias and Etgen, 1993). Furthermore, testosterone regulates mRNA levels for $\alpha_{2B}$-adrenoceptors in the rat kidney (Gong et al., 1995). Thus, there is some evidence that $\alpha_2$-adrenoceptor function can be influenced by sex and/or gonadal steroids; however, little is known about the effect of sex or gonadal steroids on $\alpha_2$-adrenoceptor function in vascular smooth muscle.

In the isolated rat tail artery, postjunctional $\alpha_2$-adrenoceptors cause contractile responses only when another vasoconstrictor is present (McGrath et al., 1990). Thus, contractile responses to a selective $\alpha_1$-adrenoceptor agonist are potentiated by the addition of an $\alpha_2$-adrenoceptor agonist, although the $\alpha_2$-adrenoceptor agonist does not cause contraction by itself (Xiao and Rand, 1989; Tsai et al., 1993). Similarly, $\alpha_2$-adrenoceptor antagonists significantly decrease contractile responses to a non-selective $\alpha$-adrenoceptor agonist, such as norepinephrine. We have shown the rat tail artery to be a useful model in investigating sex differences in vascular function (Li et al., 1997).

Therefore, the purpose of this study was to determine whether a sex-related difference exists in the function of

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postjunctional $\alpha_2$-adrenoceptors in the rat tail artery and whether circulating testicular or ovarian sex hormones are responsible for creating and/or maintaining these differences.

## 2. Materials and methods

Three-month old male and female Fischer-344 rats, weighing $300 \pm 25$ and $150 \pm 20$ g, respectively, were obtained from the NIH–NIA colony operated by Harlan Sprague–Dawley (Indianapolis, IN). Animals were kept for a minimum of 1 week before study in the University of California, Irvine Animal Resource Facility at 24°C with a 12-h light–dark cycle. Gonadectomy operations were performed under anesthesia with ketamine (90 mg/kg) and xylazine (10 mg/kg) using aseptic surgical techniques. Orchidectomy of males was performed by making a small incision across the scrotal sac and removing both testes. Females were ovariectomized by making a small incision in the lower abdomen and removing both ovaries. After gonadectomy, animals were maintained for at least 4 weeks before euthanasia. At the time of experiment, control and gonadectomized animals were approximately 4 months old.

### 2.1. Tissue preparation

After euthanizing the animals by decapitation, the tail artery was dissected out and cleaned. The proximal region was cut into eight 3-mm segments that were mounted on two wires placed through the lumen. The mounted arterial segment was then submerged in a tissue bath filled with oxygenated Krebs' solution at 37°C. The composition of the Krebs' solution was (in mM): NaCl, 122; KCl, 5.2; $CaCl_2$, 1.6; $KH_2PO_4$, 1.2; $NaHCO_3$, 25.5; $MgSO_4$, 1.2; disodium ethylenediaminetetraacetic acid, 0.027; and dextrose, 11.5. Tissue segments were equilibrated for 45 min and then stretched slowly to a resting tension of 1.0 g for males and 0.8 g for females, which were previously determined to be optimal for force development.

### 2.2. Measurement of vasoconstrictor responses

The experimental protocol used in this study was replicated from the work of Tsai et al. (1993). Tissue contraction was recorded using Gould Statham UC2 or WPI FORT 10 force transducers and a MacLab System (World

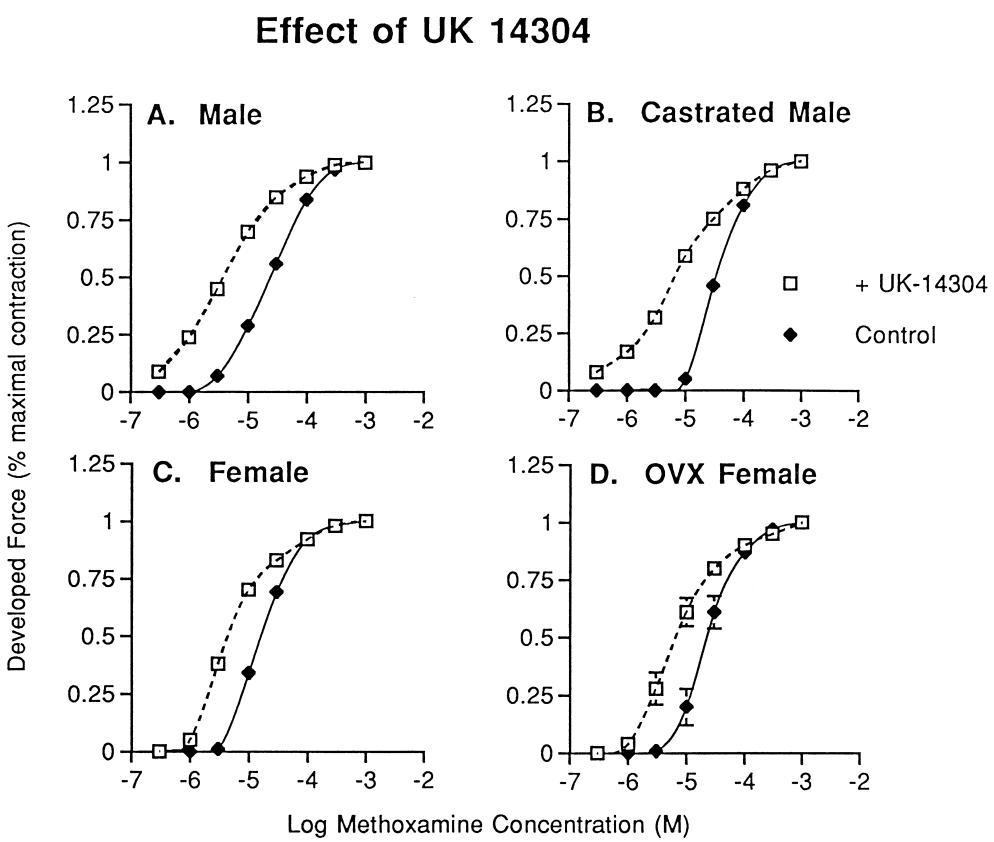


Fig. 1. Effect of addition of the $\alpha_2$-adrenoceptor agonist, UK-14304, on contractile responses to the $\alpha_1$-adrenoceptor agonist, methoxamine. Responses to arteries from (A) intact males, (B) castrated males, (C) intact females and (D) ovariectomized (OVX) females are shown. Developed force as percent of the maximal contraction to methoxamine is plotted as a function of methoxamine concentration in the absence and presence of UK-14304 (300 nM). Values are means ± S.E.M., $n = 3–5$.

Precision Instruments, New Haven, CT) to input data onto MacChart v.3.3.7. For studies of antagonist response, control concentration–response curves were constructed first by adding cumulatively increasing concentrations of (−) norepinephrine ranging from $3 \times 10^{-8}$ M to $10^{-4}$ M. Following the addition of the last concentration, tissues were washed three times with Krebs' solution, and an $\alpha_2$-adrenoceptor antagonist, either 300 nM idazoxan or 50 nM rauwolscine, was added. After equilibration for 30 min, another concentration–response curve to norepinephrine was measured in the presence of the antagonist. Only one antagonist was tested in any given arterial segment.

Alternatively, following the protocol of Tsai et al. (1993), concentration–response curves were constructed using cumulatively increasing concentrations of methoxamine, an $\alpha_1$-adrenoceptor agonist, ranging from $3 \times 10^{-8}$ M to a maximal dose of $10^{-4}$ M, in the presence of a single concentration of the $\alpha_2$-adrenoceptor agonist, UK-14304 [5-bromo-6-(2-imidazoline-2yl)-aminol-quinoxaline] (300 nM). After completion of the first concentration–response curve in the presence of UK-14304, tissues were washed and equilibrated for 30 min. Control methoxamine concentration–response curves were then determined by cumulative addition of methoxamine. Finally, together with UK-14304, an $\alpha_2$-adrenoceptor antagonist (300 nM idazoxan) was added. After 30 min equilibration, a third concentration–response curve to methoxamine was measured.

### 2.3. Drugs used

Norepinephrine bitartrate and methoxamine were supplied by Sigma (St. Louis, MO). Rauwolscine HCl and idazoxan HCl were supplied by Research Biochemicals (Natic, MA). UK-14304-18 was kindly provided by Pfizer Central Research (Sandwich, UK). Ketamine was purchased from Fort Dodge Laboratories, Fort Dodge, IA and xylazine from Miles, Shawnee Mission, KS.

### 2.4. Data analysis

Means ± S.E.M. are reported, and *n* values indicate the number of animals in each sample group. To quantify pharmacological drug potencies in the presence of different $\alpha_2$-adrenoceptor agonists or antagonists, $EC_{25}$ values (effective concentration of methoxamine or norepinephrine to produce 25% of the maximal effect) were determined with or without $\alpha_2$-adrenoceptor agonists or antagonists present. $EC_{25}$ values were selected because shifts in concentration–response curves produced by $\alpha_2$-adrenoceptor agonists and antagonists were most marked at lower norepinephrine or methoxamine concentrations. Concentration ratios were calculated from $EC_{25}$ values by dividing the $EC_{25}$ value in the presence of an $\alpha_2$-adrenoceptor agonist or antagonist by its respective control value. Concentration ratios were compared among groups using one-way analysis of variance and Fisher's protected least significant difference post-hoc test. Level of significance was chosen as $P < 0.05$.

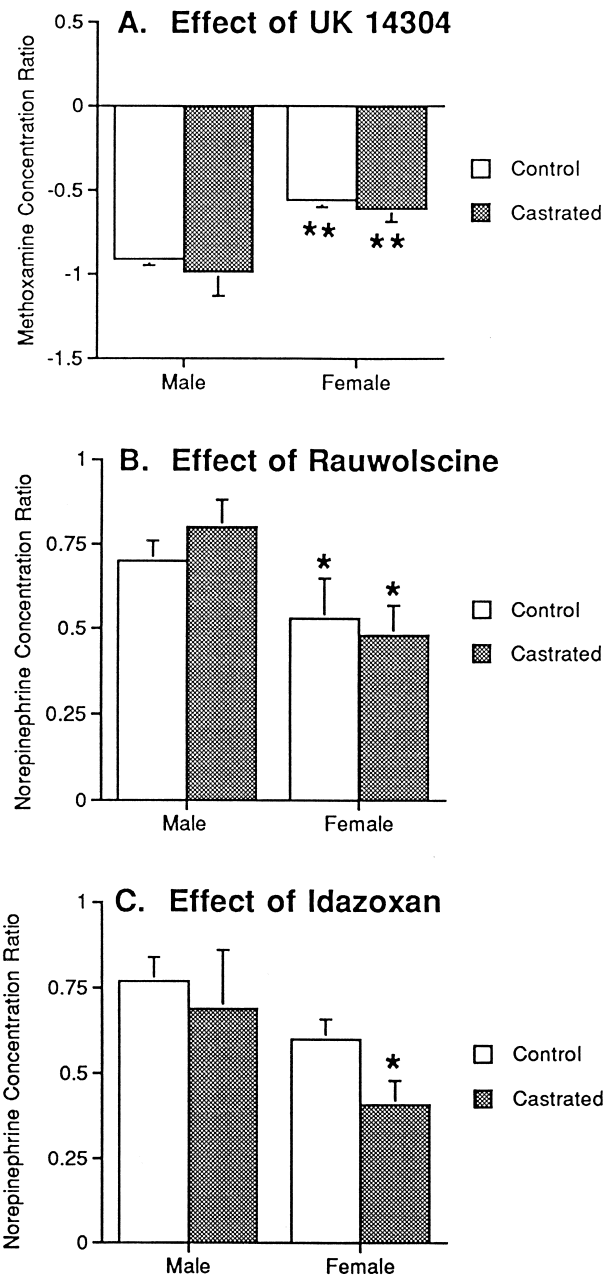


Fig. 2. Concentration ratios for agonist and antagonist treatments for arteries from males and females, intact and gonadectomized. Effects of (A) 300 nM UK-14304, (B) 50 nM rauwolscine and (C) 300 nM idazoxan are shown. $EC_{25}$ values (effective concentration of methoxamine or norepinephrine to produce 25% of the maximal effect) were determined with or without $\alpha_2$-adrenoceptor agonists or antagonists present. Concentration ratios were then calculated from $EC_{25}$ values by dividing the $EC_{25}$ value in the presence of an $\alpha_2$-adrenoceptor agonist or antagonist by its respective control value. Values are means ± S.E.M., $n = 3$–6. * Significantly different from one other group by ANOVA; ** Significantly different from two other groups.

## 3. Results

### 3.1. Effect of $\alpha_2$-adrenoceptor agonist

As shown in Fig. 1, UK-14304 (300 nM) enhanced vasoconstrictor responses to the selective $\alpha_1$-adrenoceptor agonist, methoxamine, confirming the functional effects of $\alpha_2$-adrenoceptors in the rat tail artery. Addition of UK-14304 by itself, however, had no contractile effect. As shown in Fig. 1A and C, the effect of UK-14304 was greater in tail arteries from males as compared to females. The ability of UK-14304 to enhance contractile responses to methoxamine could be blocked with addition of the $\alpha_2$-adrenoceptor antagonist, idazoxan (300 nM). Methoxamine dose ratios at the $EC_{25}$ level in the presence of UK-14304 and idazoxan were $-0.01 \pm 0.06$ in arteries from males and $0.11 \pm 0.08$ in arteries from females in contrast to the significant dose ratios in the presence of UK-14304 alone shown in Fig. 2. The ability of idazoxan to block effects of UK-14304 confirmed that $\alpha_2$-adrenoceptors were involved in the action of UK-14304 as shown previously (Tsai et al., 1993). There were no significant differences in the potency of methoxamine to cause contraction when arteries from males and females were compared. Control $EC_{25}$ values (and confidence interval) for methoxamine were: males, $6.3 \times 10^{-7}$ M ($4.6–8.5 \times 10^{-7}$) and females: $5.6 \times 10^{-7}$ M ($3.1–10 \times 10^{-7}$).

Because shifts in the concentration–response curve to methoxamine caused by UK-14304 were more pronounced at lower methoxamine concentrations, the effect of UK-14304 was quantitated by determining $EC_{25}$ values with and without UK-14304 present. Using these values, methoxamine concentration ratios were calculated. As shown in Fig. 2A, when concentration ratios for treatment with UK-14304 were compared, the effect of UK-14304 was seen to be significantly greater in arteries from males as compared to females.

Gonadectomy was performed to determine whether gonadal sex hormones maintain these sex-related differences in $\alpha_2$-adrenoceptor response. As shown in Fig. 1, 1 month after orchiectomy of males or ovariectomy of females, effects of UK-14304 did not substantially differ from the respective control animals. When these effects were quantitated by calculation of methoxamine concentration ratios

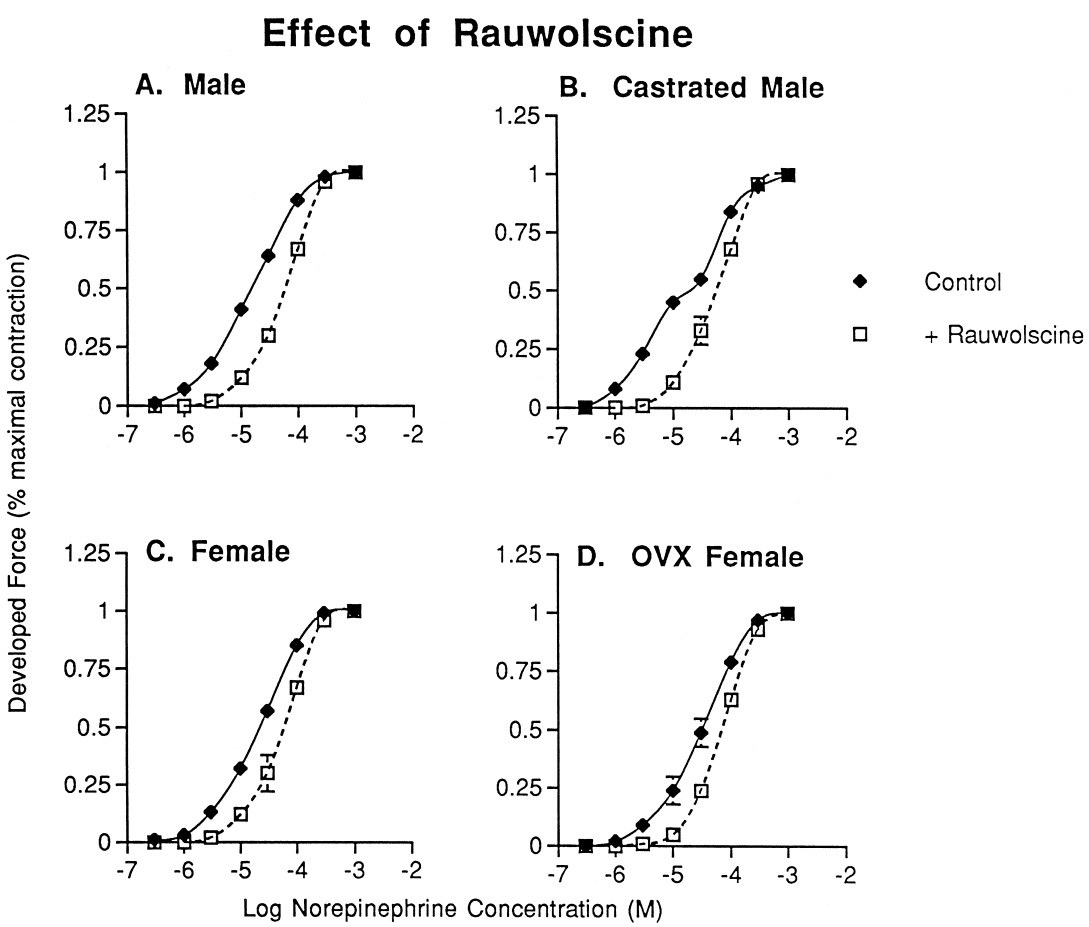


Fig. 3. Effect of addition of the $\alpha_2$-adrenoceptor antagonist, rauwolscine, on contractile responses to the non-selective adrenoceptor agonist, norepinephrine. Responses to arteries from (A) intact males, (B) castrated males, (C) intact females and (D) ovariectomized (OVX) females are shown. Developed force as percent of the maximal contraction to norepinephrine is plotted as a function of norepinephrine concentration in the absence and presence of rauwolscine (50 nM). Values are means ± S.E.M., $n = 4–6$.

(Fig. 2A), there were no significant differences in concentration ratios for UK-14304 after gonadectomy in arteries from either males or females. However, concentration ratios for UK14304 treatment in arteries from ovariectomized females were significantly different than concentration ratios in arteries from both males and orchiectomized males.

### 3.2. Effect of $\alpha_2$-adrenoceptor antagonists

Vasoconstrictor responses of the tail artery to the non-selective α-adrenoceptor agonist, norepinephrine, were significantly decreased by the addition of either rauwolscine (50 nM) (Fig. 3) or idazoxan (300 nM), $\alpha_2$-adrenoceptor antagonists. As shown by concentration ratios for the antagonists (Fig. 2B, C), effects of the antagonists tended to be greater in arteries from males, as compared to females. This reached statistical significance for treatment with rauwolscine for arteries from females as compared to castrated males (Fig. 2B). Concentration–response curves to norepinephrine itself were not significantly different when tail arteries from males and females were compared.

Gonadectomy had no significant effect on norepinephrine concentration ratios after treatment with rauwolscine or idazoxan in arteries from either males or females (Fig. 2B, C; Fig. 3). However, arteries from ovariectomized females showed concentration ratios for rauwolscine that were significantly different than arteries from orchiectomized males (Fig. 2B). In the case of idazoxan, arteries from ovariectomized females showed concentration ratios significantly different from arteries of intact males (Fig. 2C).

## 4. Discussion

This study demonstrates a male–female difference in $\alpha_2$-adrenoceptor-mediated vasoconstriction that persists in the absence of gonadal hormones. The contribution of postjunctional $\alpha_2$-adrenoceptors to vascular responses cannot be directly measured in vitro; effects of $\alpha_2$-adrenoceptor agonists are only seen in the presence of another type of agonist (Xiao and Rand, 1989; McGrath et al., 1990). A concentration of UK-14304 (300 nM), that we had previously shown to cause no contractile effect by itself, significantly enhanced contractile responses to the selective $\alpha_1$-adrenoceptor agonist, methoxamine, revealing the presence of $\alpha_2$-adrenoceptors in the rat tail artery (Tsai et al., 1993). Similar effects of UK-14304 were shown in the present study in tail arteries from both male and female rats; however, the effect of UK-14304 was significantly greater in arteries from males as compared to females.

Receptor antagonist, idazoxan or rauwolscine, was also used to shift the concentration–response curve of the non-selective α-adrenoceptor agonist, norepinephrine, revealing activation of $\alpha_2$-adrenoceptors by the non-selective agonist (Tsai et al., 1993). We had previously determined that contractile responses to an $\alpha_1$-selective adrenoceptor agonist, methoxamine, were unaffected by addition of rauwolscine (50 nM) or idazoxan (300 nM) (Tsai et al., 1993), confirming that both antagonists are selective for $\alpha_2$-adrenoceptors. In the present study, idazoxan and rauwolscine significantly shifted norepinephrine concentration–response curves in arteries from both male and female rats. Again, these effects tended to be greater in arteries from male animals. Together, these data suggest that there are sex-related differences in the function of postjunctional $\alpha_2$-adrenoceptors, with apparently greater contractile responses in tail arteries from males.

We have previously shown sex-related differences in vascular contractile responses to neuropeptide Y (Glenn et al., 1997), the pineal hormone, melatonin, (Doolen et al., 1999), and adrenergic nerve stimulation (Li and Duckles, 1993). Contractile effects to adrenergic nerve stimulation were substantially greater in arteries from males as compared to females, even after normalization for the greater diameter and wall thickness of arteries from males (Li et al., 1997). Mechanisms underlying this sex-related difference have not been established; however, enhanced postjunctional $\alpha_2$-adrenoceptor responses in males may be a contributing factor. Sex-related differences in cerebral arterial myogenic responses to increases in pressure have also been demonstrated (Geary et al., 1998).

There are several possible explanations for sex differences in sensitivity to $\alpha_2$-adrenoceptor stimulation. One possibility would be variation in density or affinity of postjunctional $\alpha_2$-adrenoceptors. Indeed, as mentioned above, there is evidence that levels of mRNA for $\alpha_{2B}$-adrenoceptors in the kidney are influenced by testosterone (Gong et al., 1995). It is not known which of the three $\alpha_2$-adrenoceptor subtypes mediates contractile responses in the rat tail artery. We have measured levels of mRNA for $\alpha_2$-adrenoceptor subtypes in rat tail arteries using in situ hybridization (McNeill et al., 1999). Using this technique, mRNA for $\alpha_{2A}$ and $\alpha_{2C}$ subtypes was localized in vascular smooth muscle, while $\alpha_{2B}$ was undetectable. Interestingly, levels of $\alpha_{2C}$ mRNA were greater in tail arteries from females as compared to males, opposite of what we would predict from our functional studies. However, levels of mRNA in a tissue may not accurately reflect levels of functional receptor protein. Further studies would be necessary to assess whether differences in contractile responses to $\alpha_2$-adrenoceptor stimulation reflect alterations in levels of functional receptor protein or are due to functional differences in other aspects, such as intracellular signalling mechanisms.

We have previously demonstrated that gonadectomy profoundly alters sex-related differences in rat artery function (Glenn et al., 1997; Geary et al., 1998; Doolen et al., 1999). Therefore, the effect of gonadectomy on vascular responses to $\alpha_2$-adrenoceptors was explored. However,

gonadectomy of either males or females did not result in any change in contractile responses to $\alpha_2$-adrenoceptor agonists or antagonists. This finding strongly suggests that differences in $\alpha_2$-adrenoceptor responsiveness of the rat tail artery are not maintained by circulating ovarian or testicular hormones. Alternatively, these differences in reactivity may reflect changes occurring during the process of development, as exposure to gonadal steroid hormones is known to cause sexual dimorphism and differentiation at specific times in development (Reisert and Pilgrim, 1991). Thus, sex differences in vascular $\alpha_2$-adrenoceptor reactivity may be established as a result of developmental regulation either of vascular properties and/or of other organ systems that subsequently influence cardiovascular function.

In summary, functional effects of vascular postjunctional $\alpha_2$-adrenoceptors were substantially greater in male rat tail arteries as compared to arteries from age-matched females. This was demonstrated by use of a selective $\alpha_2$-adrenoceptor agonist and $\alpha_2$-adrenoceptor antagonists. Since this sex-related difference in reactivity was unaffected by gonadectomy of either males or females, the presence of gonadal steroid hormones is not necessary to maintain this sex-related difference. Rather, it is likely that differences in $\alpha_2$-adrenoceptor reactivity occur as a consequence of developmental regulation of sexual dimorphism. Whether this difference in vascular reactivity might contribute to sex-related differences in occurrence of cardiovascular disease remains to be explored.

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## References

Beale, C.M., Collins, P., 1996. The menopause and the cardiovascular system. Balliere's Clinical Obstetrics and Gynecology 10, 483–513.

Del Rio, G., Velardo, A., Zizzo, G., Marrama, P., Della Casa, L., 1993. Sex differences in catecholamine response to clonidine. Int. J. Obes. 17, 465–469.

Del Rio, G., Menozzi, R., Della Casa, L., Venneri, M.G., Zizzo, G., Marrama, P., Velardo, A., 1997. Alpha-2 adrenergic activity in perimenopausal women. J. Endocrinol. Invest. 20, 603–610.

Doolen, S., Krause, D.N., Duckles, S.P., 1999. Estradiol modulates vascular response to melatonin in the rat caudal artery. Am. J. Physiol. 276, H1281–H1288.

Du, X.-J., Dart, A.M., Riemersma, R.A., Oliver, M.F., 1991. Sex difference in presynaptic adrenergic inhibition of norepinephrine release during normoxia and ischemia in the rat heart. Circ. Res. 68, 827–835.

Geary, G.G., Krause, D.N., Duckles, S.P., 1998. Estrogen reduces myogenic tone through a nitric oxide-dependent mechanism in rat cerebral arteries. Am. J. Physiol. 275, H292–H300.

Glenn, T.C., Krause, D.N., Duckles, S.P., 1997. Vascular responses to neuropeptide Y are greater in female than male rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 355, 111–118.

Gong, G., Johnson, M.L., Pettinger, W.A., 1995. Testosterone regulation of renal $\alpha_{2B}$-adrenergic receptor mRNA levels. Hypertension 25, 350–355.

Karkanias, G.B., Etgen, A.M., 1993. Estradiol attenuates $\alpha_2$-adrenoceptor-mediated inhibition of hypothalamic norepinephrine release. J. Neurosci. 13, 3448–3455.

Li, Z., Duckles, S.P., 1993. Influence of gender on vascular reactivity in the rat. J. Pharmacol. Exp. Ther. 268, 1426–1431.

Li, Z., Krause, D.N., Doolen, S., Duckles, S.P., 1997. Ovariectomy eliminates sex differences in rat tail artery response to adrenergic nerve stimulation. Am. J. Physiol. 272, H1819–H1825.

McGrath, J.C., Dunn, W.R., Templeton, A.G.B., 1990. Physiological modulation of $\alpha$-adrenoceptors and 5-HT receptor expression in blood vessels. Blood Vessels 27, 146–152.

McNeill, A.M., Leslie, F.M., Krause, D.N., Duckles, S.P., 1999. Gender difference in levels of alpha-2 adrenoceptor mRNA in the rat tail artery. Eur. J. Pharmacol. 366, 233–236.

Reisert, I., Pilgrim, C., 1991. Sexual differentiation of monoaminergic neurons—genetic or epigenetic?. Trends Neurosci. 14, 468–473.

Tsai, H., Buchholz, J.N., Duckles, S.P., 1993. Postjunctional $\alpha_2$-adrenoceptors in blood vessels: effect of age. Eur. J. Pharmacol. 237, 311–316.

White, M.M., Zamudio, S., Stevens, T., Tyler, R., Lindenfeld, J., Leslie, K., Moore, L.G., 1995. Estrogen, progesterone and vascular reactivity: potential cellular mechanisms. Endocrine Reviews 16, 739–751.

White, C.R., Darley-Usmar, V., Oparil, S., 1997. Gender and cardiovascular disease. Trends Cardiovasc. Med. 7, 94–100.

Xiao, S.-H., Rand, M.J., 1989. $\alpha_2$-Adrenoceptor agonists enhance responses to certain other vasoconstrictor agonists in the rat tail artery. Br. J. Pharmacol. 96, 539–546.