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Alteration of vascular response to norepinephrine, calcitonin gene-related peptide, and acetylcholine in orchidectomized rats[1]

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KEY WORDS orchidectomy; calcitonin gene-related peptide; acetylcholine; norepinephrine

ABSTRACT

AIM: To determine the long-term effects of orchidectomy on vasoreactivity. **METHODS:** Orchidectomized rats with or without hormone replacement were raised 1, 2, and 4 months. Vascular responses to norepinephrine, calcitonin gene-related peptide (CGRP), and acetylcholine were tested on three different arteries (thoracic aorta, pulmonary artery, and caudal artery) by functional experiments. **RESULTS:** Responses to norepinephrine, CGRP, and acetylcholine were not changed by 1 or 2 months deprivation of androgen. In aorta, the dose-response curve of CGRP was shifted to the left by 1 month of testosterone replacement. Response to norepinephrine was increased 4 months after orchidectomy and reversed by testosterone replacement in thoracic aorta. Orchidectomy did not affect CGRP- and acetylcholine-induced relaxation in all the arteries 4 months after orchidectomy. **CONCLUSION:** Compared to estrogen, the protective effects of androgen on vasoresponsiveness may be through inhibiting vasoconstriction, but is relatively weak.

INTRODUCTION

The risk of developing various cardiovascular diseases is thought to depend on gender and circulating levels of sex hormones. The protective effect of estrogen is widely accepted, as many inspiring studies have shown that besides its direct vasorelaxant effect, short-term use of 17-beta-estrodiol results in decreasing vasoconstriction response to norepinephrine and angiotensin II, as well as enhancing endothelium-dependent vasodilation induced by acetylcholine and A23187. Estrogen replacement enhances the hypotensive effect of calcitonin gene-related peptide (CGRP)[1]. Apart from the benefits on vasoreactivity, estrogen also improves lipid profile, fibrinolysis, and vascular inflammation[2].

Whether androgen protects or damages vasculature remains controversial. On the one hand, it is now commonly believed that male sex is an independent risk factor for cardiovascular diseases. Testosterone upregu-lates expression of thromboxane A_2 receptor[3], augments potassium chloride and phenylephrine induced contraction of coronary artery[4], and inhibits vasodilator response in rabbit aorta[5]. On the other hand, testosterone has an acute vasodilative effect on the rat aorta, which is partly dependent on endothelium[6], and it also depresses the constriction induced by potassium chloride, vasopressin and norepinephrine in caudal artery[7]. In a castrated cholesterol-fed rabbit

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ral androgens inhibit aortic atherosclerosis partly through lipid mediated effect[8]. Studies with different animal models and time-courses suggest that both endogenous estrogen and testosterone regulate the vasoreactivity of acetylcholine[9-11]. However, the long-term effects of androgen deprivation on vasoreactivity of CGRP and other agents have not been reported previously.

In the present study, we assessed the long-term effects of orchidectomy in various arteries of orchidectomized rats, using functional experiments of isolated thoracic aorta, pulmonary artery, and caudal artery. Acetylcholine is thought to cause an endothelium-dependent relaxation in most vessels, and the role of endothelium in mediating the vasodilator activity of CGRP varies among different blood vessels. For example, the relaxant effect of CGRP is attenuated by removal of the endothelium in isolated rat aortic rings and pulmonary artery, but not in the caudal artery rings [12-14].

MATERIALS AND METHODS

Materials Calcitonin gene-related peptide (α-rCGRP, CGRP-I, rat amino acid sequence) was from Peninsula Lab Inc (Torrance, CA). Acetylcholine, phenylephrine, norepinephrine, yohimbine, propranolol, desemethylimipramine, and normetanephrine were purchased from Sigma Chemical Company (St Louis, USA). Testosterone propionate was obtained from the ninth pharmaceutical factory of Shanghai.

Animal model Healthy male Wistar rats (Grade II, certificate № 017), 180-220 g, obtained from the Experimental Animal Laboratory of Health Science Center of Peking University were housed in wire-mesh cages at temperature room with 22 °C±5 °C, a 12:12 h light-dark cycle, supplemented with food and water *ad libitum*. All the procedures were approved by Institutional Authority for Laboratory Animal Care and adhered to the guidelines of Health Science Center of Peking University.

The experiment was divided into 3 groups. (1) Rats in the control group were sham operated. (2) Rats in the orchidectomy group were bilaterally orchidectomized at 8 weeks of age. (3) The testosterone propionate replacement group contained randomly selected rats from orchidectomized rats. Testosterone propionate replacement began 14 d after orchidectomy. To raise serum testosterone levels to the range normally found in intact adult male rats, testosterone propionate 1 mg/kg (in 5 μL Tween-20, 5 μL absolute ethanol, and 190 μL water) substitution was given sc once daily until sacrifice. The plasma levels of testosterone of the animals were measured at the end of experiment. The data showed dramatic decrease of plasma testosterone levels after orchidectomy that returned to the normal level after hormone replacement.

Preparation of isolated artery rings Rats from each group were anaesthetized with urethane (1 g/kg, ip) and killed at 1, 2, and 4 months after orchidectomy. After attaining complete anesthesia, the indicated arteries were quickly removed and placed in ice cold modified Kreb-ringer-bicarbonate (KRB) solution (mmol/L): NaCl 120, KCl 5.48, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 20, glucose 10, NaH_2PO_4 1.2, edetic acid 0.03, aerated with 95 % O_2/5 % CO_2. The blood vessels were dissected free of connective tissue and cut into rings about 3-4 mm length. The rings were mounted in organ baths containing 5 mL of KRB solution bubbled with 95 % O_2/5 % CO_2 at 37 °C. Extreme care was taken to protect the endothelium lining from inadvertent damage. More than 30 min after equilibration, the aortic and pulmonary artery rings were put under a resting tension of 1 g, and the rings from caudal arteries were given a preload of 200 mg.

Measurement of contractile and relaxant responses Contractile and relaxant responses were measured isometrically using force transducers (TB611T, Nihon Kohden Corp, Shanghai) and recorded on a polygraph recorder system (RM6000, Nihon Kohden Corp, Shanghai). The rings were pre-stimulated with 100 nmol/L norepinephrine to reach the maximum contraction for two times. Then the functional integrity of endothelium was checked in each preparation by exposing the rings pre-contracted with 10 nmol/L norepinephrine to 100 nmol/L acetylcholine. Only those rings showing relaxation of more than 70 % were selected for the study. As for rings from caudal arteries, this step was omitted, because CGRP induces an endothelium-independent vasodilation response in this artery[14]. Normetanephrine (1 μmol/L), desemethylimipramine (0.1 μmol/L), propranolol (1 μmol/L), and yohimbine (1 μmol/L) were added into the KRB solution to block the uptake and metabolism of norepinephrine and to block the β- and α_2-adrenergic receptors. The contractile-response relationship of norepinephrine was examined by applying norepinephrine in increasing concentrations in a cumulative manner. Then phenylephrine (30-300 nmol/L) was added to each preparation to approximately produce 50 % maximal contraction. When the vascu-

lar tone had reached a stable plateau, rCGRP was added to the baths at 1 % of the bath volume in a cumulative manner (from 0.01 nmol/L to 300 nmol/L). The relaxation-response curve of acetylcholine was done using the same procedure as rCGRP. Between each curve, the previous drugs were washed out by incubation with KRB for more than 30 min. At that time, the vascular tone had returned to baseline.

Statistics The results were expressed as mean±SD. Non-linear regression analysis was calculated using GraphPad Prism software. The data were analyzed using one-way ANOVA and further analyzed using the Student-Newman-Keuls (S-N-K) test for multiple comparisons between treatment groups, $P<0.05$ was considered significant.

RESULTS

Vasoconstrictor responses to norepinephrine The effects of orchidectomy and testosterone propionate replacement on norepinephrine-induced contraction were complex. Orchidectomy for 1 and 2 months did not alter the responsiveness to norepinephrine in the thoracic aorta, pulmonary artery, and caudal artery (Tab 1). However, after 4 months of castration, the dose-contraction curve of norepinephrine in thoracic aorta shifted to the left (EC_{50} for sham operated, orchidectomized, and testosterone propionate replaced rats were 35 nmol/L±17 nmol/L, 24 nmol/L±10 nmol/L, and 63 nmol/L±29 nmol/L, respectively, $P<0.05$, Tab 1, Fig 1A). In pulmonary artery the EC_{50} value remained unchanged (Fig 1B). As for caudal artery, no change was found 4 months after orchidectomy (Tab 1).

Vasodilator responses to CGRP and acetylcholine Orchidectomy for 1-4 months did not alter the response to CGRP in all the arteries tested (Tab 2). The relaxation responses to CGRP in thoracic aorta and pumonary artery were unchanged after 4 months of orchidectomy (Fig 2). As for acetylcholine-induced relaxation, also no change was found after 1, 2, and 4

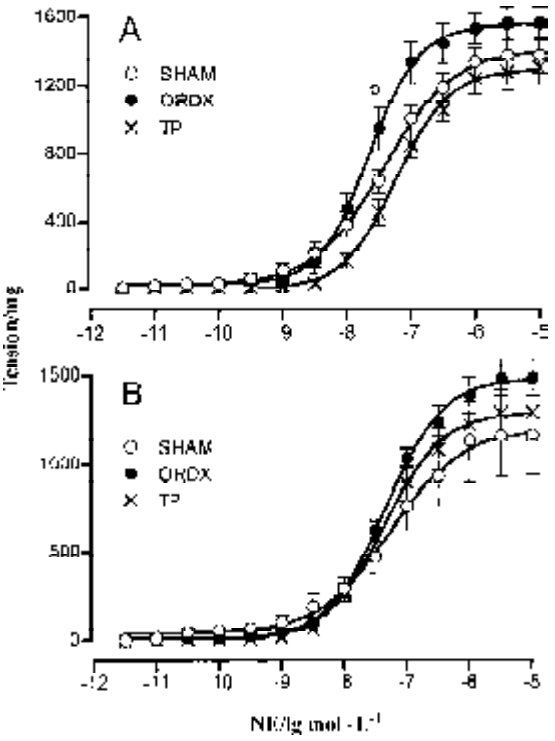

Fig 1. Long term (4 months after treatment) effects of orchidectomy (ORDX) and testosterone propionate replacement (TP) on norepinephrine (NE)-induced contraction in thoracic aorta (A) and pulmonary artery (B). *n* =5-7. Mean±SD. [b]*P*<0.05 *vs* Sham group.

Tab 1. EC_{50} value of norepinephrine-induced contraction in three kinds of arteries after 1, 2, and 4 months of castration. Mean±SD. [b]*P*<0.05 *vs* Sham group.

	Group	EC_{50} of norepinephrine/nmol·L^{-1} 1 month	*n*	2 months	*n*	4 months	*n*
Thoracic aorta	Sham	15±14	5	15±15	5	35±17	5
	ORDX	21±14	6	39±27	4	24±10[b]	5
	TP	28±17	7	29±22	6	63±29	6
Pulmonary artery	Sham	35±49	4	35±49	4	56±23	5
	ORDX	15±22	4	36±15	6	44±17	7
	TP	34±38	4	86±89	7	49±30	7
Caudal artery	Sham	254±261	5	254±261	5	788±595	4
	ORDX	577±323	5	672±330	5	301±170	6
	TP	158±109	6	715±1147	7	266±198	7

Tab 2. EC_{50} value of CGRP-induced relaxation in three kinds of arteries after 1, 2, and 4 months of castration. Mean±SD. [b]*P*<0.05 *vs* Sham group.

	Group	EC_{50} of CGRP/nmol·L^{-1}					
		1 month	*n*	2 months	*n*	4 months	*n*
Thoracic aorta	Sham	1.9±0.6	6	1.9±0.6	6	0.4±0.6	5
	ORDX	1.6±0.9	7	0.025±0.025	5	0.9±0.7	5
	TP	0.6±0.3[b]	8	0.5±0.9	6	0.8±1.6	6
Pulmonary artery	Sham	2.0±0.6	5	2.0±0.6	5	2.4±0.9	5
	ORDX	1.4±0.7	5	1.8±0.6	5	3.7±1.5	7
	TP	2.9±2.0	4	1.9±1.5	6	3.7±2.6	7
Caudal artery	Sham	24±17	4	24±17	4	3.4±1.6	4
	ORDX	4.4±6.6	3	7.3±2.4	5	6.2±3.9	6
	TP	4.0±0.7	4	11±4	5	6.6±2.7	7

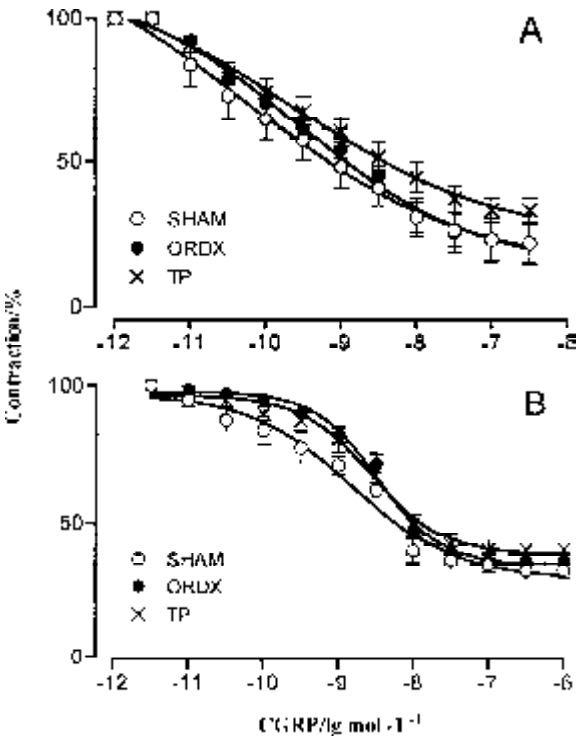

Fig 2. Long-term (4 months after treatment) effects of orchidectomy (ORDX) and testosterone propionate replacement (TP) on CGRP-induced relaxation in thoracic aorta (A) and pulmonary artery (B). *n* =5-7. Mean±SD.

months of castration (Tab 3).

DISCUSSION

Our results suggested that androgen deprivation might increase vasoconstriction to norepinephrine and had no effect on response to acetylcholine and CGRP. The present study represents the first report of vasoreactivity changes in orchidectomized rats for as long as 4 months. Most of the animal models used in previous studies are gonadectomized for only 1-4 weeks [4]. Since it is not clear how to match the life span of rats with that of human beings, especially in the old age, we think that the longer duration of gonadectomy in an animal model may represent the actual changes in human being better.

To investigate the importance of sex hormones in cardiovascular diseases and their effects on vasculature, previous studies have focused on two aspects: one is the regulatory effect of sex hormones on the production and release of vasoactive agents, another is the regulation of vascular reactivity. In our previous studies, we have found that CGRP release was upregulated 1 month after ovariectomy, but it fell back after 2 months[15]. Castration also increased CGRP release from mesenteric artery bed along with the content of CGRP in dorsal root ganglia after 1 month followed by return after 2 months and decrease after 4 months[16]. Our previous data also demonstrated that long-term decline of estrogen level decreased the endothelium-dependent vasodilation response to CGRP and acetylcholine. Estrogen replacement can reverse these alterations.

Compared with estrogen, there are few studies about the roles of androgen in the cardiovascular system, especially the interaction with vasoactive agents. The long-term effects of orchidectomy on vascular sensitivity to CGRP and other vasoactive agents have not been studied in detail. There are few reports support-

Tab 3. EC_{50} value of acetylcholine-induced relaxation in two kinds of arteries after 1, 2, and 4 months of castration. Mean±SD.

	Group	EC_{50} of acetylcholine/nmol·L^{-1}					
		1 month	*n*	2 months	*n*	4 months	*n*
Thoracic aorta	Sham	24±28	5	25±28	5	27±27	5
	ORDX	22±27	8	33±35	5	25±19	5
	TP	21±23	7	65±66	6	32±21	5
Pulmonary artery	Sham	39±25	4	39±25	4	78±119	5
	ORDX	72±31	4	58±38	6	67±54	6
	TP	61±33	4	81±103	6	84±39	6

ing the antiatherosclerotic effects of androgen, in which the role of lipid is given intensive consideration[8,17]. Our data show enhanced vasoconstriction in response to norepinephrine and no change of relaxation after long-term of orchidectomy in thoracic aorta. This is also in consistent with the previous report that treatment of nandrolone for 1-3 months reduces the contractile responses in aorta[18]. Our results give rise to a new possibility that endogenous androgen may protect vasculature by depressing vasoconstriction. It is uncertain that whether this effect is mainly through modulation of smooth muscle cells or also involves endothelium. Testosterone receptor is mainly found in smooth muscle cells[3,19] and testosterone influences the function of smooth muscle cells. Honda H *et al*[20] have studied the acute vasodilatory effect of testosterone on aorta of normotensive rats, in which the role of endothelium is excluded. However, others have reported that endogenous testosterone may modulate vascular tone through endothelium-dependent mechanisms[21].

There are also studies point to the enhancement of vasocontraction in coronary artery[4], increase of intracellular calcium in smooth muscle cells[22], and promotion of smooth muscle cell proliferation by testosterone[23]. Some clinical studies conclude that testosterone deprivation can improve endothelium-dependent relaxation[24]. Taken together, the effect of androgen is complex and may vary with different arteries and different species.

In summary, endogenous androgen shows beneficial effect on norepinephrine-induced contraction but does not influence the relaxant response to CGRP and acetylcholine. Since chronic deprivation or administration of sex hormones can influence blood vessel reactivity, they may contribute to the alteration of vascular response to vasoactive agents under pathophysiological conditions, such as atherosclerosis and hypertension in the aging.

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