Mechanisms for Vascular Effects of Androgens in Normotensive and Hypertensive Rats

O. V. Semyachkina-Glushkovskaya, T. G. Anishchenko, A. S. Kuznetsova, Ya. V. Kuznetsova, O. A. Bibikova, and A. A. Sjestnova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 2, pp. 154-157, February, 2012
Original article submitted October 4, 2010

Castration had no effect on baseline BP and vascular sensitivity to acetylcholine and deficiency of nitric oxide and prostacyclin in normotensive specimens. Castration of hypertensive specimens decreased BP and potentiated the hypotensive effects of acetylcholine, but did not modulate vascular sensitivity to the blockade of nitric oxide and prostacyclin synthesis. The removal of the testicles abolished the pressor influence of glybenclamide in hypertensive and, particularly, in normotensive males. These data indicate that the non-endothelial vascular effects of androgens (*i.e.*, stimulation of K_{ATP} channels) predominate under normal conditions. The activating effects of androgens on K_{ATP} channels decrease during hypertension, which is accompanied by inhibition of endothelium-dependent vasorelaxation. The production of nitric oxide and prostacyclin remains unchanged under these conditions. Our results suggest that endothelium-derived hyperpolarizing factor is involved in these processes.

Key Words: *androgens; hypertension; endothelial vasorelaxation; K_{ATP} channels*

The phenomenon of sex-related differences in the pathogenesis of cardiovascular diseases, including arterial hypertension, attracts much attention and remains one of the key problems in cardiology. The advantage of females over males in the gender problem is mainly related to the cardioprotective effect of estrogens [2]. The role of male sex hormones in cardiovascular resistance to hypertension is poorly understood. One the one hand, some experimental studies showed that androgens contribute to BP elevation in spontaneously hypertensive rats [12]. On the other hand, clinical observations revealed that hypotestosteronemia is typical of hypertensive men [13]. These discrepancies indicate that further studies are required to solve the problem. Little is known about the vascular effects of androgens. The majority of *in vitro* studies were performed on normotensive animals with testosterone in supraphysiological doses [8,11,15]. The contradictory results of previous studies are associated with the use of various methodical approaches. It makes difficult to understand the mechanisms for the vascular effects of androgens under normal conditions and during hypertension.

Here we studied the effect of castration on baseline BP and mechanisms of endothelial and non-endothelial vasorelaxation in normotensive (NR) and hypertensive rats (HR).

MATERIALS AND METHODS

Experiments were performed on intact (40 NR and 37 HR) and castrated (38 NR and 38 HR) male outbred albino rats weighing 250-280 g. Castration was performed by the standard method. The rats were examined in 10 days after the surgery.

Hypertension in rats was induced by the method of Goldblett with modifications [3]. Further studies were conducted 7 weeks after application of a clamp. The animals with laparotomy served as the control for HR. The mean BP was continuously monitored with a

Department of Human and Animal Physiology, N. G. Chernyshevskii Saratov State University, Russia. ***Address for correspondence:*** glushkovskaya@mail.ru. O. V. Semyachkina-Glushkovskaya

catheter on a PowerLab/400 ML401 multichannel measuring-and-computing complex and Chart 4 software (ADInstruments Ltd.). The surgeries were performed under nembutal anesthesia (40 mg/kg intraperitoneally; Sigma). Acetylcholine chloride (0.3 μg/kg intravenously; Pharma) was injected alone or after the blockade of NO synthase (L-NAME, 10 mg/kg intravenously; Sigma) and prostacyclin (indomethacin, 5 mg/kg intravenously; Sopharma) to study the mechanisms of endothelium-dependent vasorelaxation. K_{ATP} channels were blocked with glybenclamide (5 mg/kg intravenously; ALSI).

The results were analyzed by Wilcoxon test, Mann–Whitney test, ANOVA-2, and Duncan test. The differences were significant at $p<0.05$.

RESULTS

Baseline BP in NR remained unchanged after gonadectomy (Fig. 1). The removal of the testicles in HR was accompanied by a significant decrease in baseline BP as compared to that in intact HR (Fig. 1). These data indicate that the action of androgens on baseline BP (pressor influences) is manifested during hypertension, but not under normal conditions.

Acetylcholine injection to intact animals produced a hypotensive effect. The hypotensive response in HR was much lower than in NR (Fig. 2), which is consistent with the results of our previous studies [4]. The data show that endothelial vasorelaxation is suppressed in HR. Gonadectomy had a modulatory effect on vascular sensitivity to acetylcholine in HR, but not in NR. The hypotensive effects of acetylcholine differed insignificantly in intact and castrated NR (Fig. 2, *a*). By contrast, castration in HR was accompanied by a more significant decrease in BP under the influence of acetylcholine (as compared to that in intact HR; Fig. 2, *b*).

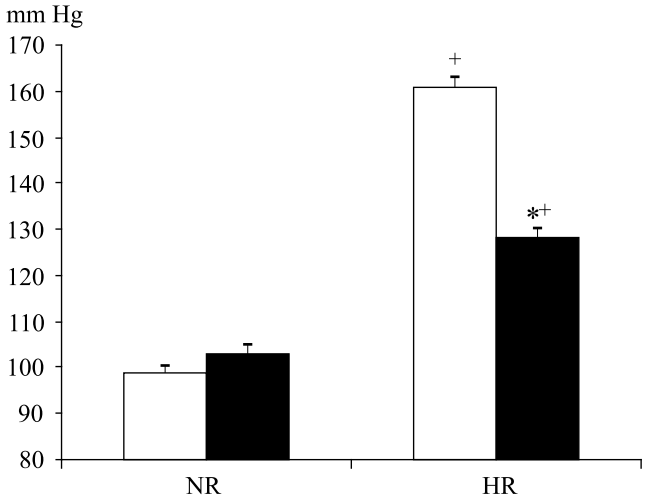

Fig. 1. Effect of castration on baseline BP in NR and HR. Light bars, intact males; dark bars, castrated males. $p<0.05$: *compared to intact males; +compared to normotensive males.

Therefore, androgens do not modulate activity of endothelial factors that stimulate vasorelaxation under normal conditions. This conclusion is confirmed by the results of *in vitro* studies on various types of vessels in NR. It was shown that the vascular effects of testosterone remain unchanged after the removal of the endothelium [8,11,15]. The reduced vascular reactivity to acetylcholine in HR significantly increases under conditions of sex hormone deficiency. Hence, the inhibitory effects of androgens on endothelial vasorelaxation are manifested during hypertension.

In the next series, we studied changes in the activity of endothelial factors that stimulate vasodilation

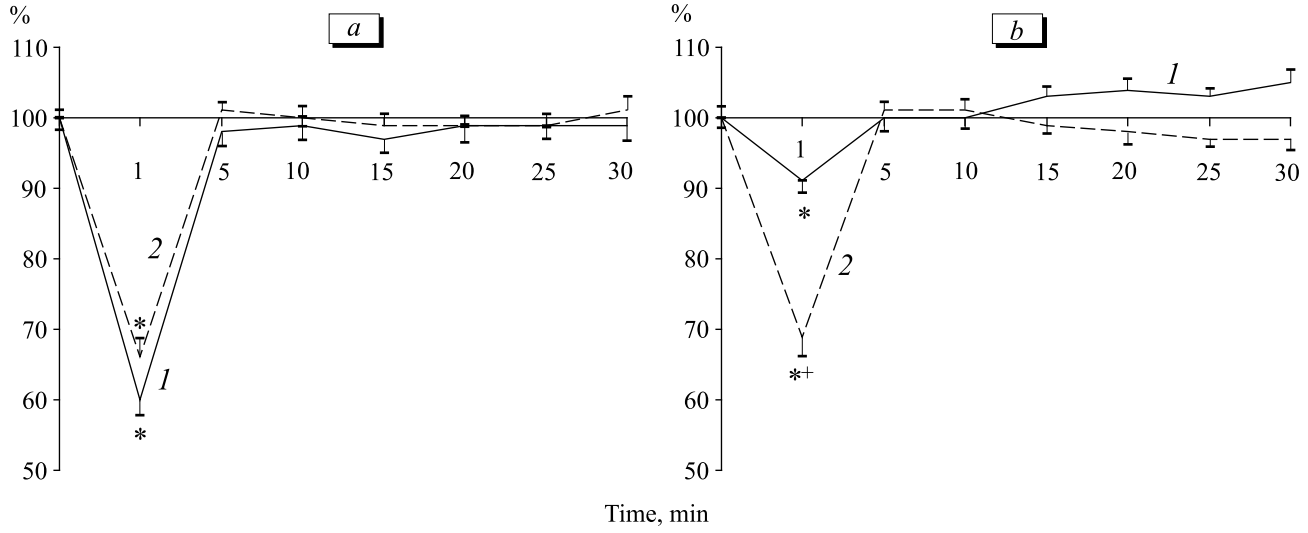

Fig. 2. Effect of castration on BP variations in NR (*a*) and HR (*b*) after acetylcholine administration. Here and in Fig. 3: intact males (*1*); castrated males (*2*). $p<0.05$: *compared to the baseline level; +compared to intact animals.

under conditions of hypertension. The role of male sex hormone in these processes was evaluated.

Experiments on intact animals showed that vascular sensitivity to NO deficiency after L-NAME administration is much greater in NR than in HR (14-18 and 7-10%, respectively; $p<0.05$). These data are consistent with the results of our previous studies showing that the development of hypertension is accompanied by reduction of NO generation [1]. Indomethacin injection was followed by the pressor influences, whose amplitude did not differ in animals of two experimental groups (17-22 and 19-23%, respectively; $p<0.05$). Therefore, prostacyclin production is not impaired in HR.

Castration of NR and HR did not modulate vascular sensitivity to the blockade of NO and prostacyclin synthesis. The data indicate that androgens do not produce a direct effect on the generation of NO and prostacyclin under normal conditions and during hypertension.

In the final series, we studied changes in non-endothelial mechanisms of vasorelaxation during hypertension. Moreover, we evaluated the contribution of male sex hormones to these processes.

The blockade of K_{ATP} channels with glybenclamide in intact rats was accompanied by the increase in BP. BP in NR was higher than in HR (Fig. 3). Castration was shown to decrease significantly vascular sensitivity to glybenclamide, particularly in NR. The hypertensive response under conditions of sex hormone deficiency after glybenclamide treatment was reduced in NR and HR (by 2.7 and 1.8 times, respectively, $p<0.05$; Fig. 3).

These data indicate that castration decreases the vascular effects of glybenclamide in HR and, particularly, in NR. Therefore, androgens have a stimulatory effect on functional activity of K_{ATP} channels, which is reduced during hypertension. The stimulatory effects of testosterone on K_{ATP} channels were observed in *in vitro* experiments on large vessels (*e.g.*, aorta) [1] and in *in vivo* studies on coronary arteries [6].

Our results indicate that the effects of castration on BP and vascular sensitivity to modulatory factors depend on the baseline physiological state and are manifested differently under normal conditions and during hypertension. The baseline parameters of BP remain unchanged in NR with sex hormone deficiency. Endothelium-dependent vasorelaxation and activities of the NO-ergic and prostanoid systems do not change in these animals. However, functional activity of K_{ATP} channels decreases significantly under these conditions. These data indicate that the non-endothelial effects of androgens on the vascular wall predominate under normal conditions. One of the mechanisms for these effects is the stimulation of K_{ATP} channels. The involvement of other types of K_{ATP} channels in this process cannot be excluded. We showed that the activity of endothelial factors is not modulated by androgens under normal conditions. These factors probably compensate reduced activity of K_{ATP} channels in castrated specimens, thus maintaining the basal BP during androgen deficiency.

Castration of HR has antihypertensive effects, which is manifested in a decrease of BP and potentiation of endothelium-dependent vasorelaxation after acetylcholine treatment. Previous studies showed that NO, prostacyclin, and endothelium-derived hyperpolarizing factor are involved in the hypotensive effects of acetylcholine [5]. Our experiments revealed that castration of HR does not modulate vascular sensitivity to the blockade of NO and prostacyclin. It can be suggested that an acetylcholine-induced increase in endothelial vasodilation under these conditions is as-

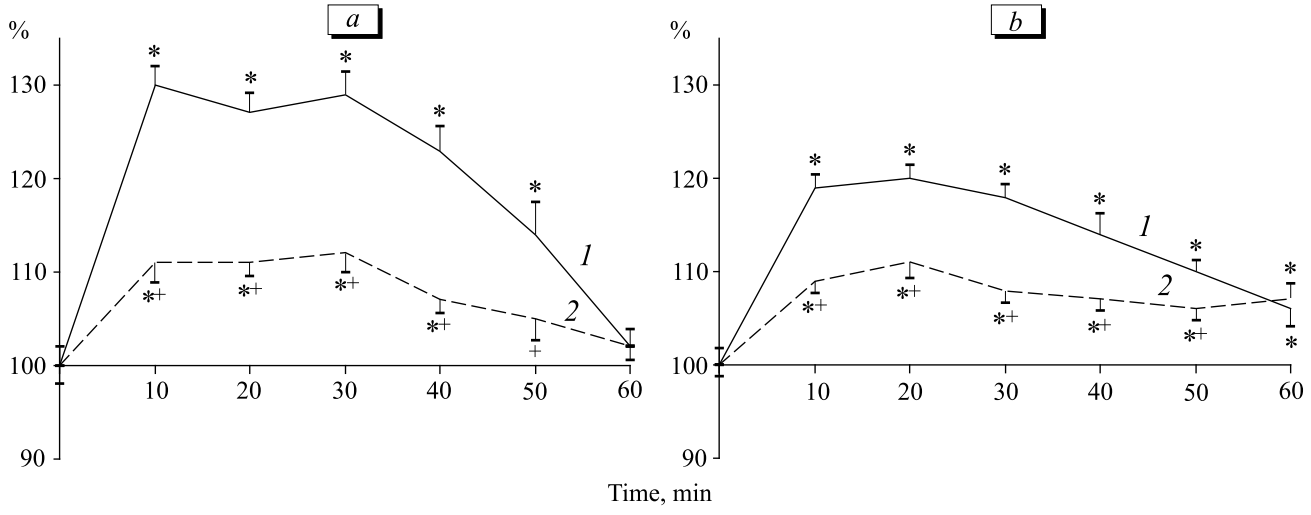

Fig. 3. Effect of castration on BP variations in NR (*a*) and HR (*b*) after glybenclamide administration.

sociated with activation of endothelium-derived hyperpolarizing factor. The inhibition of this factor is considered as a main pathogenetic factor of hypertension [10]. Gonadectomy in HR decreases vascular sensitivity to glybenclamide. However, the observed changes in HR are less pronounced than in NR. This treatment abolishes the effect of androgens on K^+ channels in HR, which is probably related to the reduced production of male sex hormones during hypertension [13]. Recent studies showed that K_{ATP} channels play an important role in the activation of endothelium-derived hyperpolarizing factor [7,9]. Our results and published data suggest that ineffective stimulation of K_{ATP} channels by androgens in hypertensive specimens contributes to the decrease in vascular sensitivity to acetylcholine. We conclude that the inhibition of endothelium-dependent vasorelaxation during hypertension most likely serves as a consequence, but not as a cause of hypertension in males.

This work was supported by the Federal Target Program "Innovation Personnel of Russia" for 2009-2012 (state contract No. P1063).

REFERENCES

1. O. V. Glushkovskaya-Semyachkina, T. G. Anishchenko, T. A. Sindyakova, *et al*., *Byull. Eksp. Biol. Med*., **142**, No. 7, 13-17 (2006).
2. V. I. Kobrin and E. E. Porman, *Vestn. Aritmol*., No. 1, 72-83 (2000).
3. RF Inventor's Certificate No. 68280. Device for the Modeling of Experimental Renal Hypertension. O. V. Glushkovskaya and T. G. Anishchenko. *Byull. Izobret*., No. 33, 27.11.2007.
4. O. V. Semyachkina-Glushkovskaya, T. G. Anishchenko, V. A. Berdnikova, *et al*., *Byull. Eksp. Biol. Med*., **146**, No. 7, 33-37 (2008).
5. R. Busse, G. Edwards, M. Feletou, *et al*., *Trends Pharmacol. Sci*., **23**, No. 8, 374-380 (2002).
6. T. Chou, K. Sudhir, S. Hutchison, *et al*., *Circulation*, **94**, No. 10, 2614-2619 (1996).
7. H. Coleman, M. Tare, and H. Parkington, *Clin. Exp. Pharmacol. Physiol*., **31**, No. 9, 641-649 (2004).
8. V. P. Deenadayalu, R. E. White, J. N. Stallone, *et al*., *Am. J. Physiol. Heart Circ. Physiol.*, **281**, No. 4, H1720-H1727 (2001).
9. G. Edwards, K. Dora, and M. Gardener, *Nature*, **396**, 269-272 (1998).
10. K. Goto, K. Fujii, Y. Kansui, *et al*., *Proc. Austr. Physiol. Pharmacol. Soc*., **34**, 65-70 (2004).
11. H. Honda, T. Unemoto, and H. Kogo, *Hypertension*, **34**, No. 6, 1232-1236 (1999).
12. T. Kumai, M. Tanaka, M. Watanabe, *et al*., *Hypertension*, **26**, No. 1, 208-212 (1995).
13. P. Y. Liu, A. K. Death, and D. J. Handelsman, *Endocr. Rev*., **24**, No. 3, 313-340 (2003).
14. J. F. Reckelhoff, *Hypertension*, **37**, No. 5, 1199-1208 (2001).
15. P. Yue, K. Chatterjee, C. Beale, *et al*., *Circulation*, **91**, No. 4, 1154-1160 (1995).