

T. STYŚ, E. SZCZEPAŃSKA-SADOWSKA

**EFFECT OF NG-NITRO-L-ARGININE ON PRESSOR ACTION OF ARGININE VASOPRESSIN IN NORMOTENSIVE (WKY) AND SPONTANEOUSLY HYPERTENSIVE (SHR) RATS**

Department of Clinical and Applied Physiology, Medical Academy, Warsaw, Poland

In order to find out whether the pressor effect of arginine vasopressin (AVP) is limited by release of nitric oxide (NO) and whether it is altered in hypertension, blood pressure (MAP) and heart rate (HR) responses to i.v. administration of AVP were compared in conscious normotensive Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats under control conditions and during blockade of NO synthesis induced by i.v. administration of NG-nitro-L-arginine (L-NOARG). In control experiments AVP elicited significant elevations of MAP in WKY and SHR. The maximum increases in WKY and SHR amounted  $26 \pm 3$  and  $16 \pm 3$  mmHg, respectively, and did not differ significantly from each other. In WKY increase of MAP was associated with significant bradycardia. Administration of AVP in this strain during blockade of NO synthesis resulted in significantly smaller increase of blood pressure ( $13 \pm 5$  mmHg) than under control conditions ( $p < 0.001$ ), and in nonsignificant changes of HR. In SHR AVP caused a progressive significant decrease of blood pressure associated with transient tachycardia. The results indicate that blockade of NO synthesis does not enhance but reduces increase of blood pressure in WKY and transforms the pressor action of this peptide into the hypotensive effect in SHR. This phenomenon is discussed in relevance to the possible unfavorable effects of AVP on coronary and/or cerebral circulation during blockade of NO formation.

**Key words:** *arginine vasopressin, NG-nitro-L-arginine, nitric oxide, WKY, SHR.*

**INTRODUCTION**

It is generally accepted that arginine vasopressin (AVP) is one of the most potent vasoconstrictory agents (1), however, recent evidence indicates that AVP may also cause vasodilatation (2–5). With this regard, Katusic et al., (2) supported evidence that in some vascular beds the vasodilatory effect of AVP is caused by the endothelium-derived relaxing factor (EDRF). Accumulating evidence indicates that the EDRF activity is largely mediated by the nitric oxide (NO) (6). In previous study on conscious Long Evans rats Gardiner et al. (7) reported enhancement of vasoconstricting action of AVP after blockade of NO synthesis with NG-nitro L-arginine methyl ester (L-NAME). Because disturbances in NO synthesis have been implicated in pathogenesis of

hypertension (6), the aim of the present study was to find out whether blockade of NO synthesis by i.v. administration of NG-nitro-L-arginine (L-NOARG) alters the pressor responsiveness to AVP in normotensive Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats.

## METHODS

### *Animals*

The experiments were performed on 10 male SHR and 21 male normotensive rats of the parent WKY strain (age 12 to 14 weeks), weighing 250–300 grams. They were fed a commercial laboratory chow containing 0.5% NaCl and had free access to water. One day before the experiment they were subjected to ether anaesthesia and instrumented with polyethylene catheters, introduced into the abdominal aorta and vena cava inferior for measurements of blood pressure (MAP) and heart rate (HR) and for intravenous administration of tested substances. Both catheters were filled with heparin diluted in saline (1000 u/ml) and exteriorized on the neck. After the surgery the animals were placed in individual experimental cages for recovery. The experiments were performed after 24 of 48 h following the surgery.

### *Experimental design*

At the beginning of each experiment arterial catheter was connected to the pressure transducer (Statham P23DB) and Gould amplifier. The blood pressure unit was connected through an interface to Amstrad-Schneider CPC 6128 computer which calculated on line systolic (SAP), diastolic (DAP), and mean arterial blood pressure (MAP), as well as the heart rate period (HRp, msec), which corresponded to the distance between the two subsequent peaks of the two systolic pressure waves.

Three sets of experiments were carried out:

#### *Series I*

Series I was conducted on 7 WKY in order to determine the time course of cardiovascular effects of i.v. administration of L-NOARG (NG-nitro-L-arginine, Sigma) alone. After stabilization of blood pressure and heart rate 10 mg/kg of L-NOARG was injected i.v. and MAP and HRp were monitored during subsequent 60 minutes.

#### *Series II*

Series II was performed on 7 WKY and 5 SHR to determine changes of MAP after AVP (arginine<sup>8</sup>-vasopressin, Calbiochem-Behring Corp.) administration. After 30–40 min allowed for stabilization of blood pressure and heart rate, 5 ng of AVP was administered i.v. as a bolus. Arterial blood pressure and heart rate were recorded during 20 minutes after AVP administration.

#### *Series III*

Series III was carried out on 7 WKY and 5 SHR to determine effect of AVP on MAP and HR after L-NOARG administration. After stabilization period L-NOARG (10 mg/kg) was administered i.v., as in series I, and AVP was injected i.v. 20 min after L-NOARG administration. MAP and HRp were monitored continuously before and during 40 min following L-NOARG injection.

## Statistical analysis

The results are presented as means together with their standard errors ( $\bar{x} \pm SE$ ). The effects of tested substances were analyzed by the analysis of variance (ANOVA). One way ANOVA for repeated measurements was used to evaluate within group variability and factorial ANOVA to determine differences between groups of experiments. The differences in maximum changes were tested using Student's t-test. Newman-Keuls a posteriori test was used to analyze individual differences between the individual means (8).

## RESULTS

Baseline values of MAP and HRp before administration of L-NOARG, AVP and L-NOARG+AVP are presented in *Table 1*.

*Table 2.* Baseline values of heart rate period (HRp), systolic blood pressure (SAP), mean blood pressure (MAP) and diastolic blood pressure (DAP) in WKY and SHR before administration of L-NOARG, AVP and AVP after L-NOARG

Series	Strain	HRP (msec)	SAP (mmHg)	MAP (mmHg)	DAP (mmHg)
L-NOARG	WKY	175 ± 6	160 ± 6	118 ± 4	93 ± 4
	SHR	163 ± 3	218 ± 13	169 ± 11	134 ± 9
AVP	WKY	163 ± 8	148 ± 6	114 ± 3	93 ± 3
	SHR	155 ± 4	224 ± 6	176 ± 6	142 ± 5
L-NOARG + AVP	WKY	201 ± 4	182 ± 11	143 ± 7	120 ± 6
	SHR	169 ± 8	262 ± 6	207 ± 3	170 ± 2

$\bar{x} \pm SE$  are shown

### Series I

Effect of L-NOARG on blood pressure and heart rate in WKY. Administration of L-NOARG elicited prompt significant increase of MAP [ $F(24,144) = 13.56$ ;  $P < 0.001$ ] and significant decrease of heart rate [ $F(24,144) = 4.92$ ;  $P < 0.001$ ], as indicated by significant elongation of the HRp (*Fig. 1*). Changes of MAP and HRp reached their maximum at 15 min (MAP) and 20 min (HRp) after administration of L-NOARG and stabilized at these levels during the whole observation period (*Fig. 1*). Small fluctuations of MAP and HRp during the period of stabilization were not significantly different.

### Series II

Effect of AVP administration on blood pressure and heart rate. In WKY AVP induced a significant [ $F(20,120) = 12.76$ ;  $P < 0.001$ ] 5 min lasting elevation of MAP, the maximum increase by  $26 \pm 6$  mm Hg being observed 2 min after AVP administration. Subsequently, MAP returned to the baseline value. The increase of blood pressure was associated with transient statistically

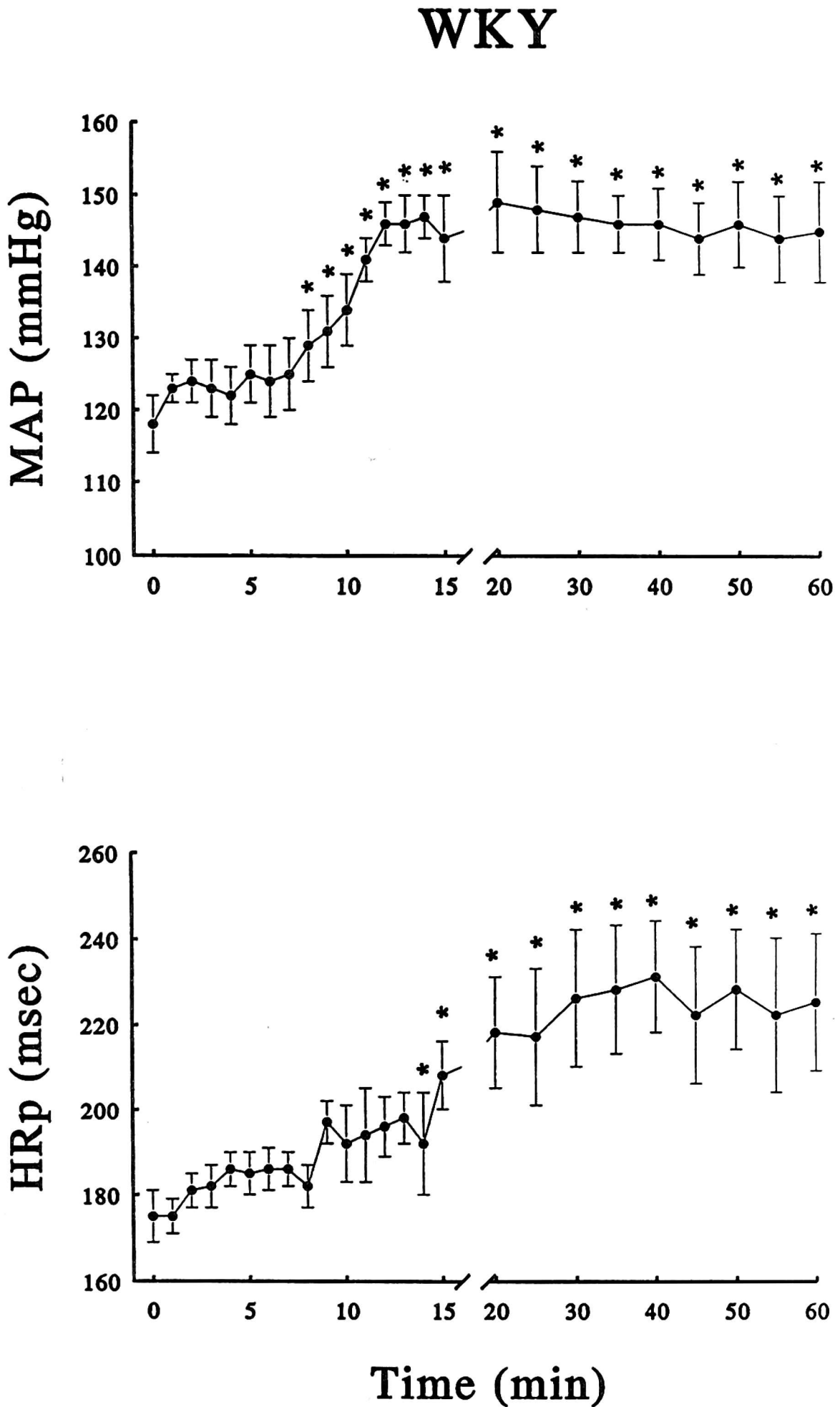


Fig. 1. Effect of i.v. administration of L-NOARG on mean arterial pressure (MAP) and heart rate period (HRp) in Wistar Kyoto rats. \* — significant difference from baseline at a level of  $P < 0.05$  or better.

## WKY

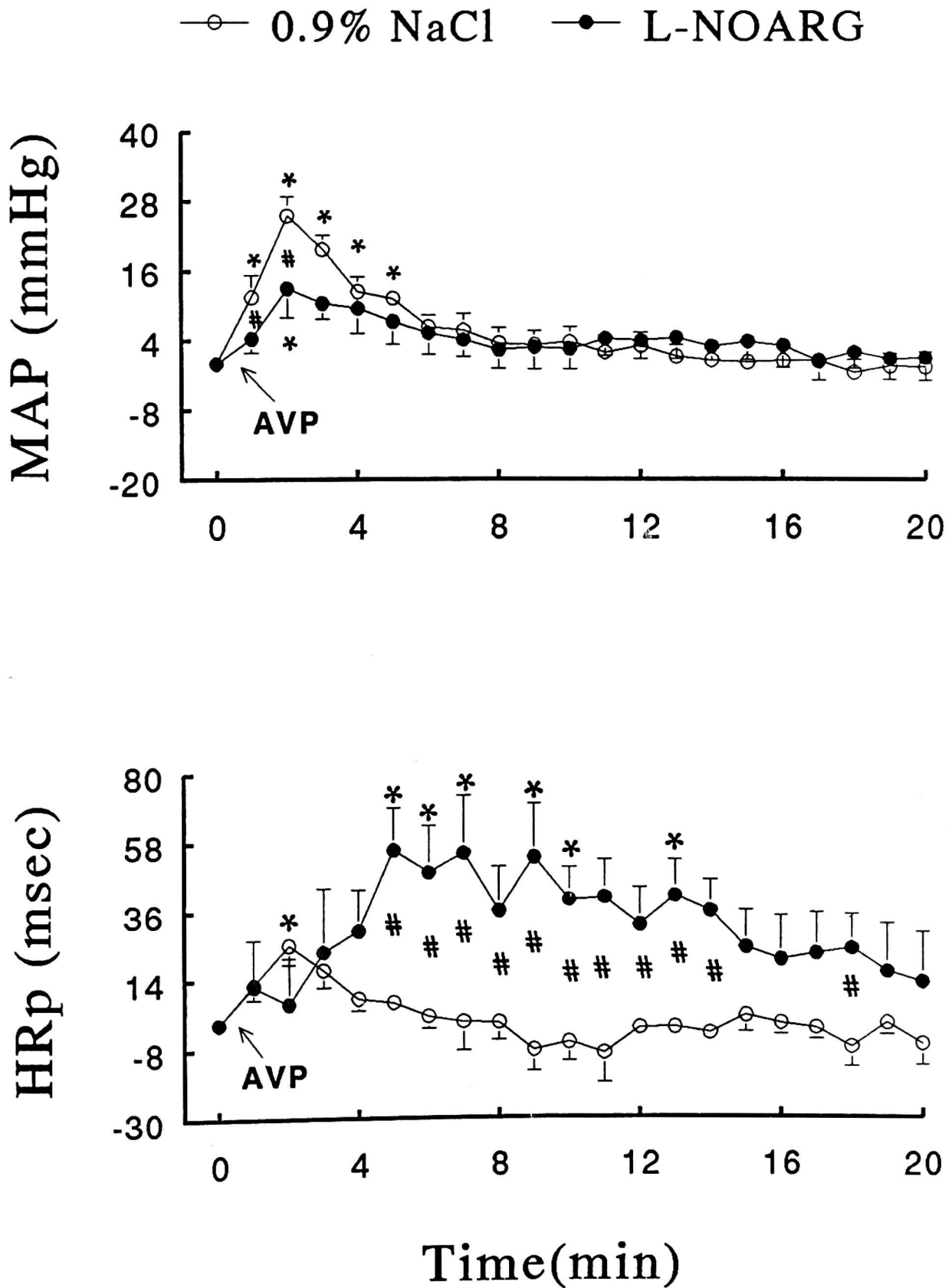


Fig. 2. Effect of administration of AVP (5 ng) on MAP (upper panel) and HRp (lower panel) under control conditions (empty circles) and during blockade of NO synthesis (black circles) in WKY. Arrow indicates time of injection of AVP. # — significant difference between control and L-NOARG experiments at a level of  $P < 0.05$  or better. For other explanations see Fig. 1.

significant bradycardia [ $F(20,120) = 3.42$ ;  $P < 0.001$ ], as shown by elongation of HRp ( $25 \pm 6$  msec) at 2 min after AVP injection (*Fig. 2*). SHR responded to arginine vasopressin with a similar, statistically significant [ $F(20,80) = 0.19$ ;  $P < 0.001$ ] increase in MAP immediately after administration, with the maximum increase of  $16 \pm 3$  mmHg. A small increase of HRp ( $12 \pm 3$  msec) did not reach the level of statistical significance (*Fig. 3*). Blood pressure increases after administration of AVP in WKY and SHR did not differ significantly.

### *Series III*

Modulation of the effects of AVP by blockade of NO synthesis. As shown in *Fig. 4* administration of L-NOARG elicited comparable significant elevation of MAP in WKY and SHR, whereas HRp increased only in WKY ( $P < 0.001$ ). The difference in change of HRp between WKY and SHR was significant ( $P < 0.05$ ). In L-NOARG treated WKY, arginine vasopressin elicited only small, though significant [ $F(20,110) = 3.61$ ;  $P < 0.001$ ], transient increase in MAP (*Fig. 2*). The whole time course of MAP changes in WKY treated with AVP alone and with AVP combined with L-NOARG administration were not different by two-way ANOVA, but the maximum increase was significantly smaller ( $P < 0.05$ ) (*Fig. 2*). A delayed significant [ $F(20,110) = 2.1$ ;  $P < 0.05$ ] bradycardia was observed between 5 and 9 min with the maximum elongation of HRp by  $53 \pm 13$  msec at 5 min following administration of AVP.

In L-NOARG treated SHR, injection of AVP elicited a progressive significant decrease of MAP [ $F(20,68) = 108.65$ ;  $P < 0.001$ ] (*Fig. 3*). At the end of experiment MAP decreased by  $23 \pm 4$  mmHg. Shortly after the experiment, three SHR exhibited dramatic decrease of blood pressure followed by sudden death. The changes of MAP in SHR treated with AVP alone and in SHR treated with AVP after L-NOARG administration were significant by two-way ANOVA [ $F(1,4) = 46.01$ ;  $P < 0.01$ ]. In comparison to controls, L-NOARG treated rats responded to AVP administration with two periods of significant acceleration of heart rate between 2 and 4 and 16–18 min (*Fig. 3*). Responses to MAP in WKY and SHR to AVP injection after L-NOARG administration were significantly different [ $F(1,10) = 25.96$ ;  $p < 0.001$ ] from each other. Similarly, significant difference was found between the two strains treated with L-NOARG and AVP with regard to HRp [ $F(1,10) = 15.06$ ;  $p < 0.001$ ].

### DISCUSSION

In agreement with the previous reports (9, 10) i.v. administration of 5 ng of AVP elicited significant increases of MAP in WKY and SHR. Similarly, blockade of NO synthesis by i.v. administration of 10 mg of L-NOARG caused

## SHR

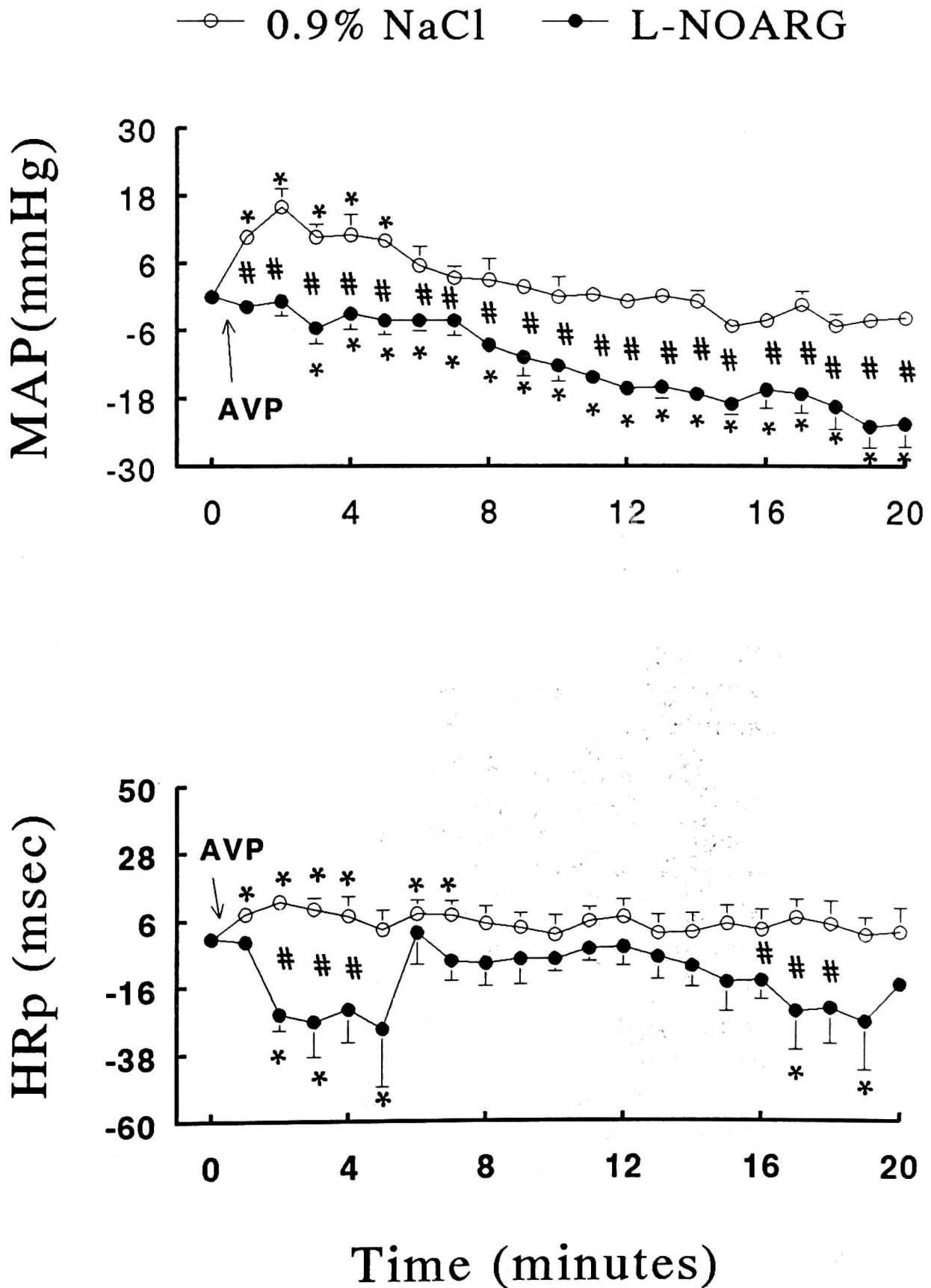


Fig. 3. Effect of administration of AVP (5 ng) on MAP (upper panel) and HRp (lower panel) under control conditions and during blockade of NO synthesis in SHR. For other explanations see Figs 1 and 2.

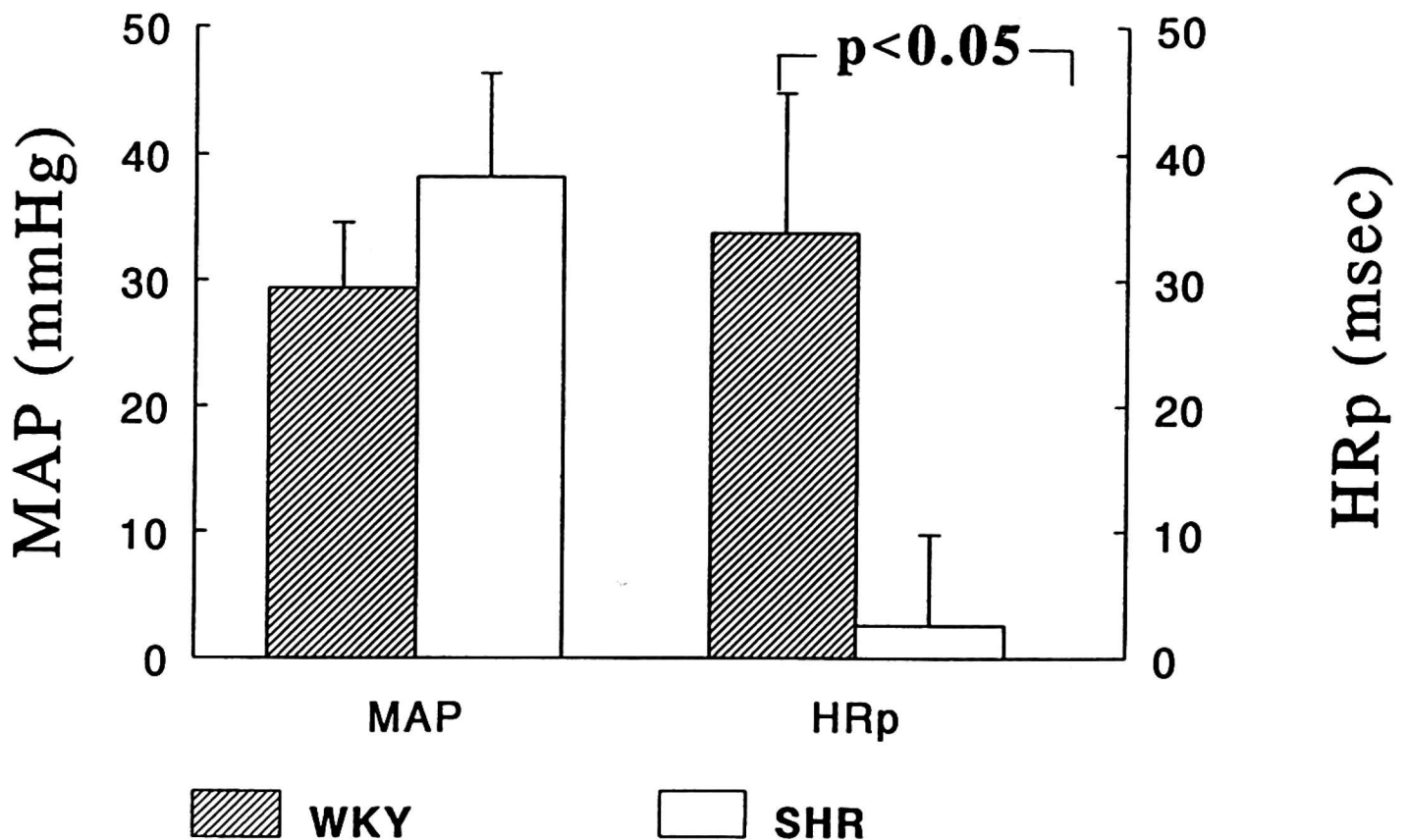


Fig. 4. Increases of mean arterial pressure (MAP) and heart rate period (HRp) after administration of L-NOARG (10 mg/kg i.v.) in Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats.

prolonged elevation of blood pressure that was similar in both strains (11). On the other hand, both treatments reduced heart rate only in WKY exerting no significant effect in SHR.

The most conspicuous finding of the present study is a significant attenuation of the pressor response to AVP in WKY and the progressive decrease of blood pressure after administration of AVP in SHR during blockade of NO synthesis. Fig. 1 indicates that in WKY AVP was injected during the period of stabilization of blood pressure. Similarly, long lasting (40 min) stabilization of blood pressure was found after administration of L-NOARG in SHR (11, and our unpublished data). Thus, it is justified to assume that in the present study AVP was applied during the period of full hemodynamic adaptation to blockade of NO synthesis.

The mechanism of attenuation of the pressor response to AVP in WKY and of the decrease of blood pressure in SHR cannot be clarified at present. The available evidence indicates that some of the vasodilatory effects of AVP are mediated by release of EDRF (2). As nitric oxide is one of the most potent endothelium derived vasodilatory factor, blockade of synthesis of this compound was expected to cause enhancement of the pressor effect of AVP, the action opposite to that observed in our study. One of the possible explanations of the present findings may be a significant potentiation of the vasoconstrictory action of AVP in the coronary circulation in the absence of the antagonizing



vasodilatory effect of this peptide, mediated by nitric oxide. Accumulating evidence indicates that AVP effectively impairs the coronary blood flow and the external work efficiency (12, 13). Thus, elimination of NO mediated vasodilatory effect in the present study could reinforce hemodynamically unfavourable effects of AVP. Especially, the decreased cardiac performance might account for the progressive decrease of blood pressure in SHR.

The other factor which may be responsible for a deleterious effect of the combination of AVP and blockade of NO synthesis in SHR might be a vasoconstriction of the cerebral blood vessels. The reports on effects of AVP on cerebral circulation are controversial, both vasoconstrictory and vasodilatory actions being described (2, 4, 14). Some evidence indicates that vasodilatory effect of vasopressin on cerebral vessels is mediated by EDRF (2). Blockade of NO synthesis has been shown to produce significant decrease of the basal cerebral blood flow (15). Thus, administration of AVP after blockade of NO formation may cause significant deterioration of the cerebral blood flow and brain metabolic functions.

An interesting finding of this study is a significant delayed bradycardia which follows administration of AVP in L-NOARG treated WKY. This phenomenon occurred when MAP returned to the level preceding AVP administration and therefore could not be secondary to AVP induced blood elevation. Its relevance to potentiation of AVP induced enhancement of the cardiac component of the baroreflex (16) during L-NOARG blockade remains to be elucidated.

*Acknowledgements:* This study was supported by a grant from the Committee of Scientific Research (KBN) and by the Medical Academy of Warsaw.

#### REFERENCES

1. Altura BM, Altura BT. Actions of vasopressin, oxytocin and synthetic analogs on vascular smooth muscle. *Fed Proc* 1984; 43, 80–6.
2. Katusic ZS, Shepherd JT, Vanhoutte PM. Vasopressin causes endothelium-dependent relaxation of canine basilar artery. *Circ Res* 1984; 55: 575–9.
3. Kim P, Katusic ZS, Vanhoutte PM. Endothelium-dependent responses to arginine vasopressin. In *Vasopressin: Cellular and Integrative Functions*. AW Cowley, Jr. JF Liard, DA Ausiello (eds). New York Raven Press, 1988, pp. 455–59.
4. Kozniwska E, Szczepańska-Sadowska E. V2-like receptors mediate cerebral blood flow increase following vasopressin administration in rats. *J Cardiovasc Pharmacol* 1990; 15: 579–85.
5. Schwartz J, Liard JF, Ott C, Cowley AW Jr. Hemodynamic effects of neurohypophyseal peptides with antidiuretic activity in dogs. *Am J Physiol* 1985; 249, (Heart Circ Physiol 18): H1001–H1008.
6. Vanhoutte PM. Endothelium and control of vascular function. *Hypertension* 1989; 13: 658–67.

7. Gardiner SM, Compton AM, Kemp PA, Bennett T. Effects of NG- nitro-L-arginine methyl ester or indomethacin on differential regional and cardiac haemodynamic actions of arginine vasopressin and lysine vasopressin in conscious rats. *Br J Pharmacol* 1991; 102, 65–72.
8. Winer BJ. *Statistical Principles in Experimental Design* (2nd edition). New York: McGraw-Hill, 1977, 46–227.
9. Łapiński M, Stępniakowski K, Januszewicz A, Noszczyk B, Szczepańska-Sadowska E. Atrial natriuretic factor enhances vasopressin-induced bradycardia in normotensive (WKY) but not in spontaneously hypertensive (SHR) rats. *Europ J Clin Invest* 1988; 18: 568–74.
10. Stępniakowski K, Łapiński M, Januszewicz A, Noszczyk B, Szczepańska-Sadowska E. Pressor responsiveness to vasopressin in spontaneously hypertensive rats. *Acta Physiol Pol* 1989; 40(2): 171–82.
11. Januszewicz A. Influence of endothelin on cardiovascular function in experimental hypertension. *Warsaw Medical Academy*, 1992. (in Polish)
12. Boyle WA, Segel LD. Direct cardiac effects of vasopressin and their reversal by vascular antagonist. *Am J Physiol* 1986; 25: H734–H741.
13. Boyle WA, Segel LD. Attenuation of vasopressin-mediated coronary constriction and myocardial depression in the hypoxic heart. *Circulation Research* 1990, 66: 710–721.
14. Nakai M. Contractile effects of perivascularly applied vasopressin on the pial artery of the cat brain. *J Physiol* 1987; 387: 441–52.
15. Koźniewska E, Osęka M, Styś T. Effect of endothelium-derived nitric oxide on cerebral circulation during normoxia and hypoxia in the rat. *J Cereb Blood Flow Metab* 1992; 12: 311–17.
16. Breeze BL, Walker BR. Vasopressinergic augmentation of cardiac baroreceptor reflex in conscious rats. *Am J Physiol* 1990; 258: R860–8.

Received: October 20, 1993

Accepted: January 7, 1994

Author's address: E. Szczepańska-Sadowska, Department of Clinical and Applied Physiology, Warsaw Medical Academy, Krakowskie Przedmieście str 26/28, 00-927 Warsaw, Poland