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EFFECT OF VASOPRESSIN AND V_1 RECEPTORS BLOCKADE ON HYPOTENSIVE ACTION OF ANP IN NORMOTENSIVE (WKY) AND SPONTANEOUSLY HYPERTENSIVE RATS.

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The aim of the study was to find out whether vasopressin (AVP) modifies hypotensive and heart rate accelerating effects of atrial natriuretic peptide (ANP) in normotensive (WKY) and spontaneously hypertensive (SHR) conscious rats. The effect of i. v. administration of 1; 2 and 4 μg of ANP on blood pressure (MP) and heart rate (HR) was compared during i. v. infusion of 0.9% NaCl (NaCl), NaCl+AVP ($1.2 \text{ ng kg}^{-1} \text{ min}^{-1}$) and NaCl+dEt₂AVP (V_1 receptors antagonist, $0.5 \mu\text{g kg}^{-1} \text{ min}^{-1}$). AVP increased MP in SHR and WKY and decreased HR in SHR. V_1 antagonist decreased MP and increased HR only in SHR. In SHR ANP decreased MP and increased HR during NaCl, AVP and V_1 antagonist infusion. In WKY these effects were observed only during AVP administration. In each experimental situation hypotension and tachycardia induced by ANP were greater in SHR than in WKY. In both strains ANP induced changes in MP and HR were enhanced during AVP in comparison to NaCl infusion. V_1 antagonist did not modify effects of ANP in WKY and SHR. The results indicate that ANP abolishes hypertensive response induced by blood AVP elevation and that the basal levels of endogenous vasopressin acting through V_1 receptors does not interfere with hypotensive action of ANP neither in WKY nor in SHR.

Key words: *Atrial natriuretic peptide, vasopressin, V_1 receptors antagonist, spontaneously hypertensive rats, normotensive rats.*

INTRODUCTION

The effects of atrial natriuretic peptide (ANP) on arterial blood pressure have been extensively investigated in animals and in human subjects with regard to its putative role as an endogenous hypotensive substance (1,2). Specifically, it has been suggested that interaction of ANP with other vasoactive substances may be important for the cardiovascular effects of this peptide (3—5). Accumulating evidence indicates that vasopressin may interact

with ANP at cellular level (6—8). In our previous study we reported that ANP enhances reflex bradycardia induced by vasopressin but does not interfere with pressor responsiveness to this peptide (4). The present study was aimed at elucidating whether prolonged elevation of blood AVP concentration or blockade of V_1 receptors will modify blood pressure and heart rate responses to ANP. Since pressor responsiveness to ANP and vasopressin is altered in hypertensive animals (4,9—12). The experiments were performed on spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats to find out whether putative interaction between vasopressin and ANP may be different in SHR.

MATERIALS AND METHODS

Animals

The experiments were performed on 20 spontaneously hypertensive (SHR) and 20 normotensive male rats of the parent WKY strain at age of 13—16 weeks, maintained on a 12 h light/dark cycle (light from 07.00 to 19.00 h). They were fed with a commercial laboratory chow (Laboratory Chow Manufacture, Morycz, Poland) containing 0.5% NaCl and had free access to water.

One or two days before the experiment they were instrumented with arterial and venous catheters enabling blood pressure measurements and i. v. administration of tested substances in conscious animals. The catheters (PE 20) were introduced under ether anaesthesia into the vena cava inferior through the right femoral artery. Both catheters were connected with polyethylene tubings (PE 60) filled with saline and heparin, plugged with stoppers, tunneled under the skin and exteriorized on the neck. The animals were placed in the individual cages for recovery.

Experimental design

During the experiment food and water were removed from the experimental cage. The arterial blood pressure catheter was connected to the blood pressure unit consisting of transducer (Statham P23Db), amplifier (Gould) and recorder (Seconic 100F). HR was determined from the pulse pressure tracing. The venous catheter was connected with a syringe pump. Hypotensive responsiveness to ANP was evaluated by determination of the magnitude of the maximum decrease in blood pressure and of duration of blood pressure reduction after administration of 1.0, 2.0 and 4.0 μg ANP. The experiments started after 40 min of rest to allow for stabilization of blood pressure and heart rate. During the experiments the animals were infused with 0.9% NaCl (20 $\mu\text{l min}^{-1}$) to maintain patency of the venous catheter.

Two series of experiments were performed.

Series 1. (10 WKY and 10 SHR) was aimed at elucidating whether prolonged elevation of blood vasopressin level modifies blood pressure and heart changes elicited by atrial natriuretic peptide.

Each experiment consisted of the control (saline) and vasopressin (saline + AVP) periods. During the control period the animals were injected with 1.0, 2.0 and 4.0 μg ANP (rat 28 ANF, Peninsula Inc., USA) administered i. v. as bolus at 20–40 min intervals in 10 μl of 0.9% NaCl during 10 s. After the last dose of ANP vasopressin (arginine⁸-vasopressin, Calbiochem-Behring Corp., USA) was added to the infusion fluid and administered at a rate of 1.2 $\text{ng kg}^{-1} \text{min}^{-1}$ until the end of the experiment. Thirty min after the start of AVP infusion injections of ANP were repeated as during the control period.

Series 2. (10 WKY and 10 SHR) was performed to find out whether blockade of V_1 receptors modifies changes of blood pressure and heart rate in response to ANP administration. The experimental design was similar as in series 1. The experiments consisted of the control (saline) and V_1 blockade periods. V_1 receptors antagonist dEt_2AVP (13) was infused at a rate of 0.5 $\mu\text{g kg}^{-1} \text{min}^{-1}$ (saline + dEt_2AVP). Effectiveness of V_1 receptors blockade was checked by injection of the pressor dose of AVP (10 ng i. v.) before and during dEt_2AVP administration of 1.0, 2.0 and 4.0 μg of ANP were repeated during saline + dEt_2AVP periods.

The position of venous catheter in the lumen of the vessel was verified by autopsy at the conclusion of the experiment.

Statistical analysis

Means and their standard errors are presented throughout the study and in Tables and Figures. The significance of differences between means was evaluated by paired t-test, factorial ANOVA, or factorial ANOVA with measurements repeated on time concluded with the Newman-Keuls a posteriori test, when appropriate (14). A probability of <0.05 was considered to be significant.

RESULTS

Resting values of mean and systolic blood pressure in *Series 1* were as follows: WKY – MP = 120 ± 3 mmHg; SHR – MP = 182 ± 5 , mmHg. Heart rate amounted to 271 ± 13 in WKY and 379 ± 16 beats min^{-1} in SHR. The differences in blood pressure and heart rate between WKY and SHR were significant at a level of $p < 0.001$.

Effects of ANP on blood pressure during saline and vasopressin infusion

Time course of MP changes and maximum changes of SP after administration of ANP during infusion of saline and AVP in WKY and SHR are presented in *Figs. 1 and 2* and *Table 1*.

During infusion of saline ANP did not elicit significant changes in MP and only slight reduction in SP in WKY. In SHR a significant decrease in MP was observed after injection of 4 μg of ANP. Systolic pressure was reduced after each dose of ANP; the maximum decrease in SP (ΔSPmax) being observed 5 min after ANP administration. The maximum changes in SP significantly exceeded those observed in WKY (*Table 1*).

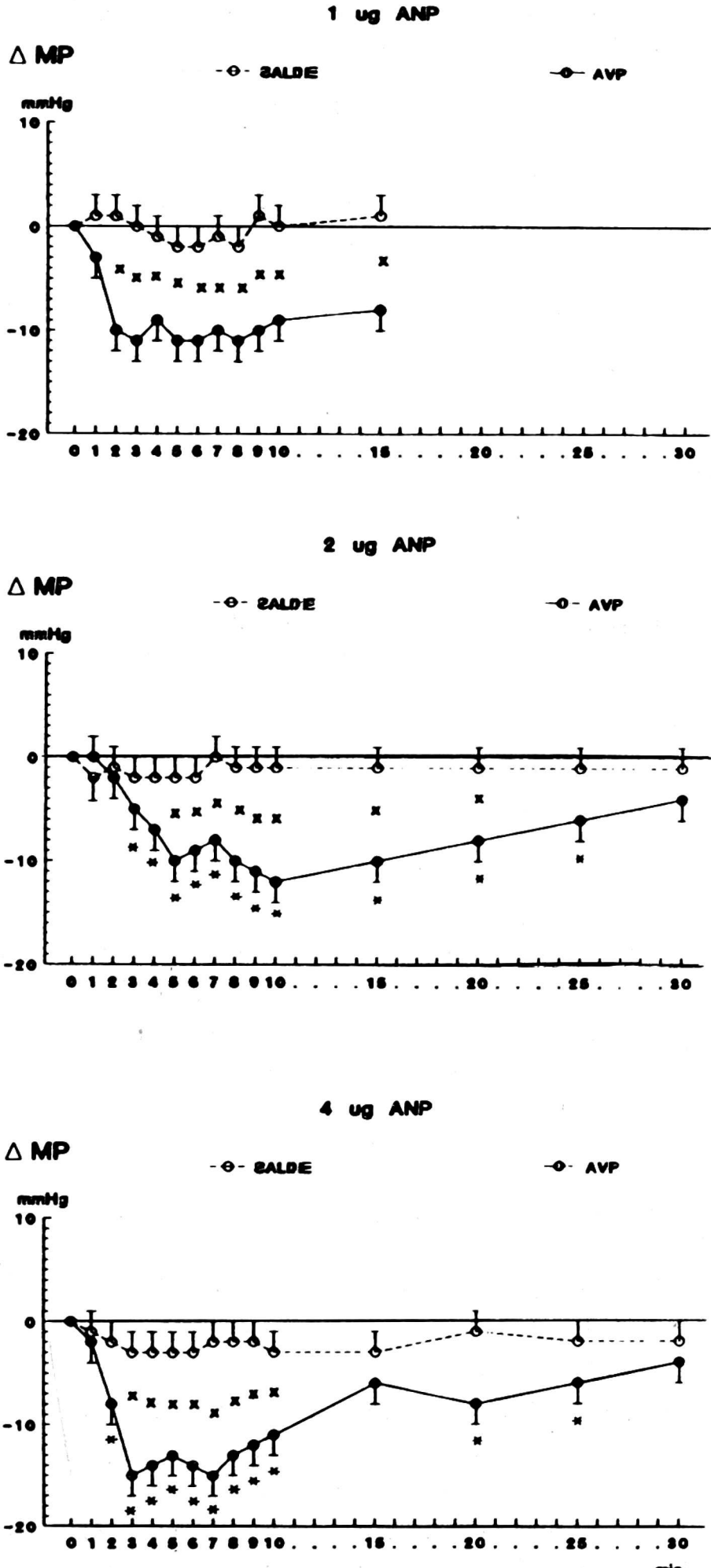


Fig. 1. Changes of mean arterial pressure (Δ MP) after i. v. injection of atrial natriuretic peptide (ANP) during infusion of saline and of vasopressin in normotensive (WKY, n = 10) rats. Asterisks (*) above and below the lines designate significant differences from the preinjection values. Symbols (x) between lines indicate significant differences between saline and vasopressin infusion. Mean \pm SE are shown. * , x — p < 0.05.

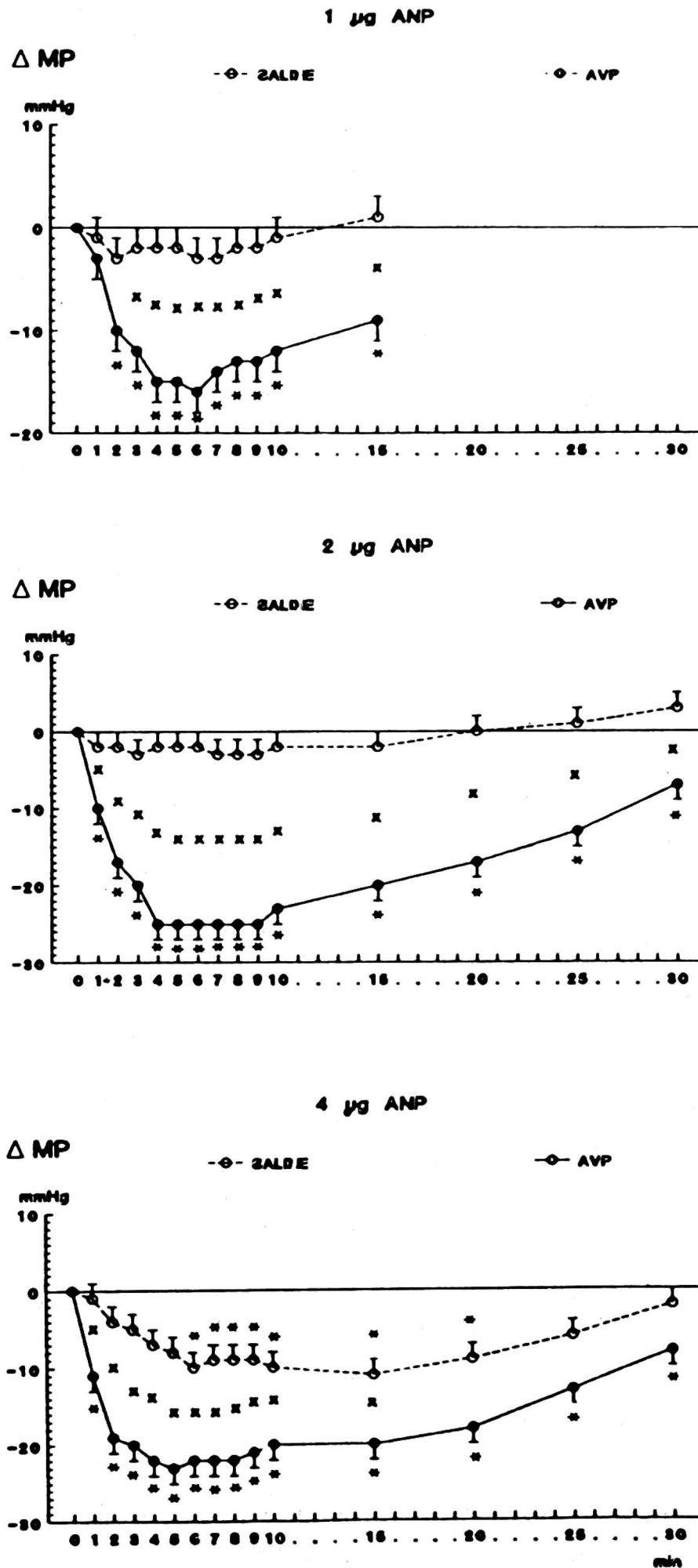


Fig. 2. Changes of mean arterial pressure (Δ MP) after i.v. injection of atrial natriuretic peptide (ANP) during infusion of saline and of vasopressin in spontaneously hypertensive (SHR, $n = 10$) rats. For symbols and abbreviations see Fig. 1.

Table 1. Maximum changes of systolic pressure (Δ SP) and of heart rate (Δ HR) after injection of ANP in spontaneously hypertensive (SHR) and normotensive (WKY) rats during infusion of 0.9% NaCl, vasopressin (AVP) and of V₁ receptors antagonist (dEt₂AVP).

			ANP (μ g)		
			1.0	2.0	4.0
Δ SP (mmHg)	SHR	NaCl (1)	$-8.5 \pm 2.0^{**}$	$-8.2 \pm 2.6^*$	$-17.4 \pm 2.2^{***}$
		AVP (2)	$-19.1 \pm 2.7^{***}$	$-31.9 \pm 4.1^{***}$	$-29.0 \pm 2.6^{***}$
	WKY	NaCl (3)	$-3.0 \pm 0.6^{***}$	$-4.2 \pm 1.5^*$	-4.6 ± 2.1
		AVP (4)	$-14.5 \pm 2.2^{**}$	$-12.7 \pm 3.3^{**}$	$-16.9 \pm 4.3^{**}$
1—2 p<0.001; 3—4 p<0.001; 1—3 p<0.01; 2—4 p<0.001					
Δ HR (b/min)	SHR	NaCl (5)	$-5.5 \pm 2.2^*$	$-5.0 \pm 1.8^*$	$-6.9 \pm 2.4^*$
		d (Et ₂) AVP (6)	$-4.3 \pm 1.2^{**}$	-1.9 ± 1.7	$-8.7 \pm 2.0^{**}$
	WKY	NaCl (7)	-3.8 ± 1.8	-1.5 ± 1.8	$-5.3 \pm 1.9^*$
		d (Et ₂) AVP (8)	$-6.8 \pm 2.4^*$	-4.5 ± 2.2	-3.7 ± 1.7
5—6 NS; 7—8 NS; 5—7 NS; 6—8 NS					
2—6 p<0.001; 4—8 p<0.001					
Δ SP (mmHg)	SHR	NaCl (1)	$17.0 \pm 5.8^*$	$31.0 \pm 7.9^{**}$	$30.0 \pm 9.9^*$
		AVP (2)	$26.0 \pm 7.2^{**}$	$59.0 \pm 8.2^{***}$	$56.0 \pm 6.4^{***}$
	WKY	NaCl (3)	1.0 ± 5.3	1.0 ± 2.3	3.0 ± 8.2
		AVP (4)	$20.0 \pm 6.2^*$	$18.0 \pm 7.4^*$	$32.0 \pm 10.7^*$
1—2 p<0.001; 3—4 p<0.001; 1—3 p<0.001; 2—4 p<0.001					
Δ HR (b/min)	SHR	NaCl (5)	$17.0 \pm 5.7^*$	7.0 ± 6.2	$25.0 \pm 5.4^{**}$
		d (Et ₂) AVP (6)	14.0 ± 7.3	$19.0 \pm 6.9^*$	$29.0 \pm 5.3^{***}$
	WKY	NaCl (7)	2.0 ± 5.9	5.0 ± 4.8	15.0 ± 6.9
		d (Et ₂) AVP (8)	1.0 ± 6.4	14.0 ± 8.7	5.0 ± 6.9
5—6 NS; 7—8 NS; 5—7 NS; 6—8 p<0.05					
2—6 p<0.001; 4—8 p<0.001					

Means \pm standard errors are shown. The asterisks indicate significant differences from resting values by Student's paired test. * p<0.05, ** p<0.01, *** p<0.001.

During AVP infusion injections of ANP resulted in significant reduction of MP and SP in both strains. In WKY the time course of mean blood pressure changes was significantly affected by 2 and 4 μ g of ANP (Figs. 1 and 2).

Similarly as during saline infusion the maximum changes in blood pressure occurred 5 min after ANP administration. In both strains changes in SP_{max} were significantly greater than during saline infusion. Each dose of ANP decreased blood pressure in WKY and SHR to the level which was observed before infusion of AVP (*Figs 1 and 2, Table 1*).

Effects of ANP on heart rate during saline and AVP infusion

During infusion of saline ANP did not significantly alter HR in WKY (*Fig. 3, Table 2*). In SHR the overall analysis of variance of time course of HR changes revealed significant elevation of HR after 2 and 4 μ g of ANP (*Fig. 4*). The maximum changes in HR (ΔHR_{max}) were significant at each dose of ANP (*Table 1*).

During AVP infusion significant acceleration of heart rate was observed after each dose of ANP in both strains (*Figs 3 and 4, Table 1*). Changes of HR followed those in blood pressure and usually attained the maximum at 5–6 min after ANP injection. Each dose of ANP injected during AVP infusion increased the HR to the level which did not differ significantly from that observed before vasopressin infusion (*Figs 3 and 4, Table 1*).

Effects of ANP on blood pressure during saline and V_1 receptors antagonist infusion

The overall analysis of variance of time course in blood pressure changes did not reveal significant changes of MP after injection of ANP in WKY during saline and dEt_2AVP infusion (data not shown). A small but significant decrease of SP was noted after 4 μ g during saline and after 1 g of ANP during dEt_2AVP infusion (*Table 1*).

In SHR changes of MP after 1, 2 and 4 μ g of ANP administered during saline infusion did not attain a level of significance in this series (*Fig. 5*). During infusion of V_1 antagonist MP was significantly reduced after 4 μ g of ANP. Significant decrements in SP were observed in SHR 5 min after injection of each dose of ANP during saline infusion and after 1 and 4 μ g during infusion of V_1 antagonist (*Table 1*). Differences between changes in MP and SP observed after injection of ANP during saline and dEt_2AVP infusion in WKY and SHR were not significant (*Fig. 5 and Table 1*).

Effect of ANP on heart rate during saline and dEt_2AVP infusion

In WKY administration of ANP did not change HR significantly neither during saline nor during dEt_2AVP infusion (*Table 1*).

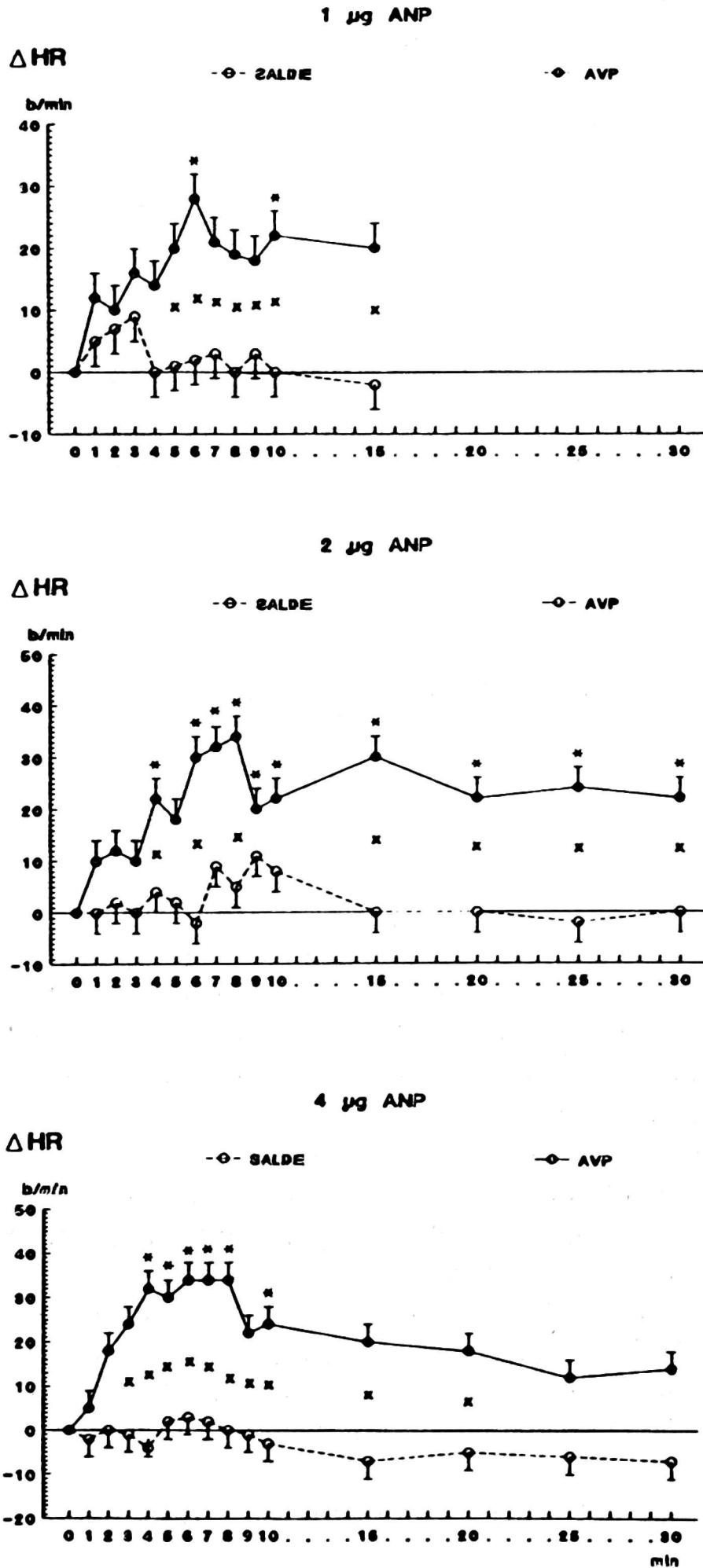


Fig. 3. Changes of heart rate (Δ HR) after i.v. injection of atrial natriuretic peptide (ANP) during infusion of saline and of vasopressin in normotensive (WKY, n = 10) rats. For symbols and abbreviations see Fig. 1.

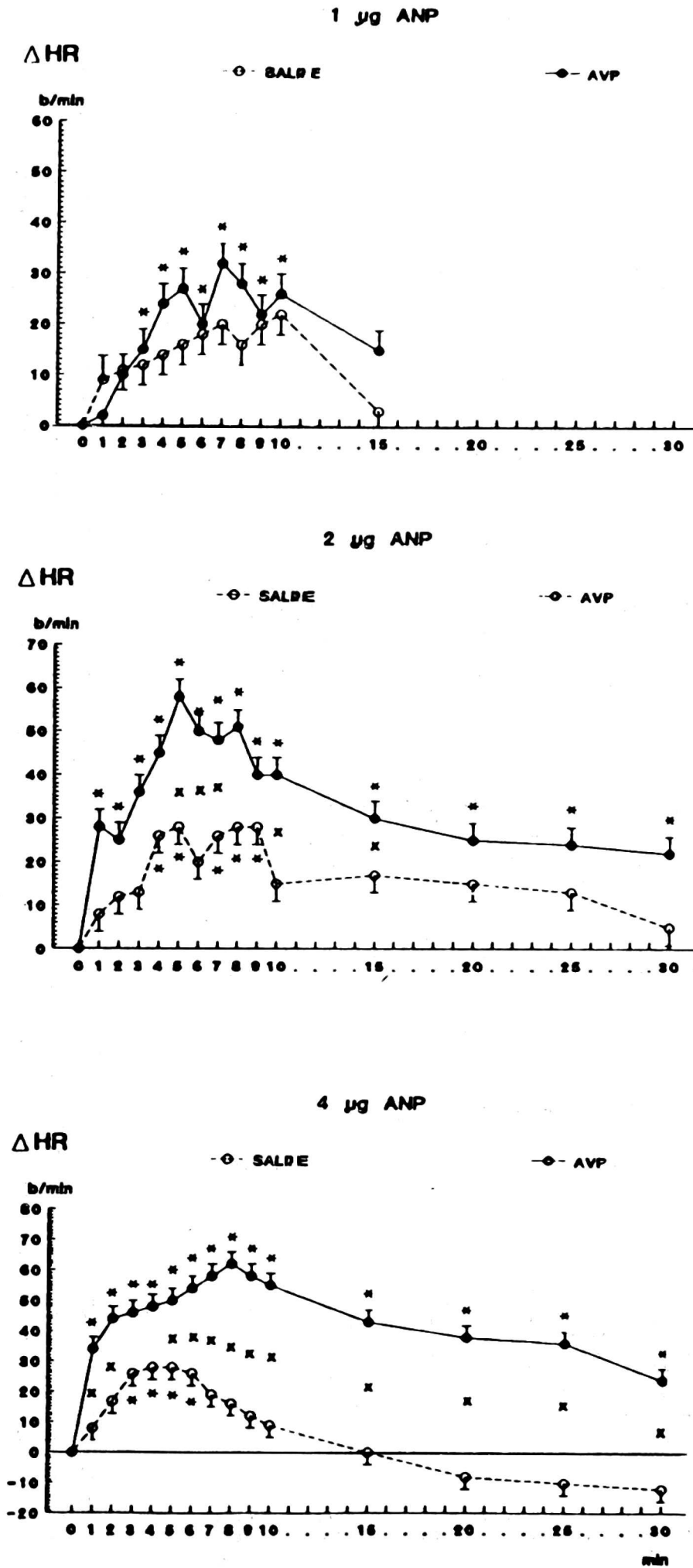


Fig. 4. Changes of heart rate (Δ HR) after i.v. injection of atrial natriuretic peptide (ANP) during infusion of saline and of vasopressin in spontaneously hypertensive (SHR, $n = 10$) rats. For symbols and abbreviations see Fig. 1.

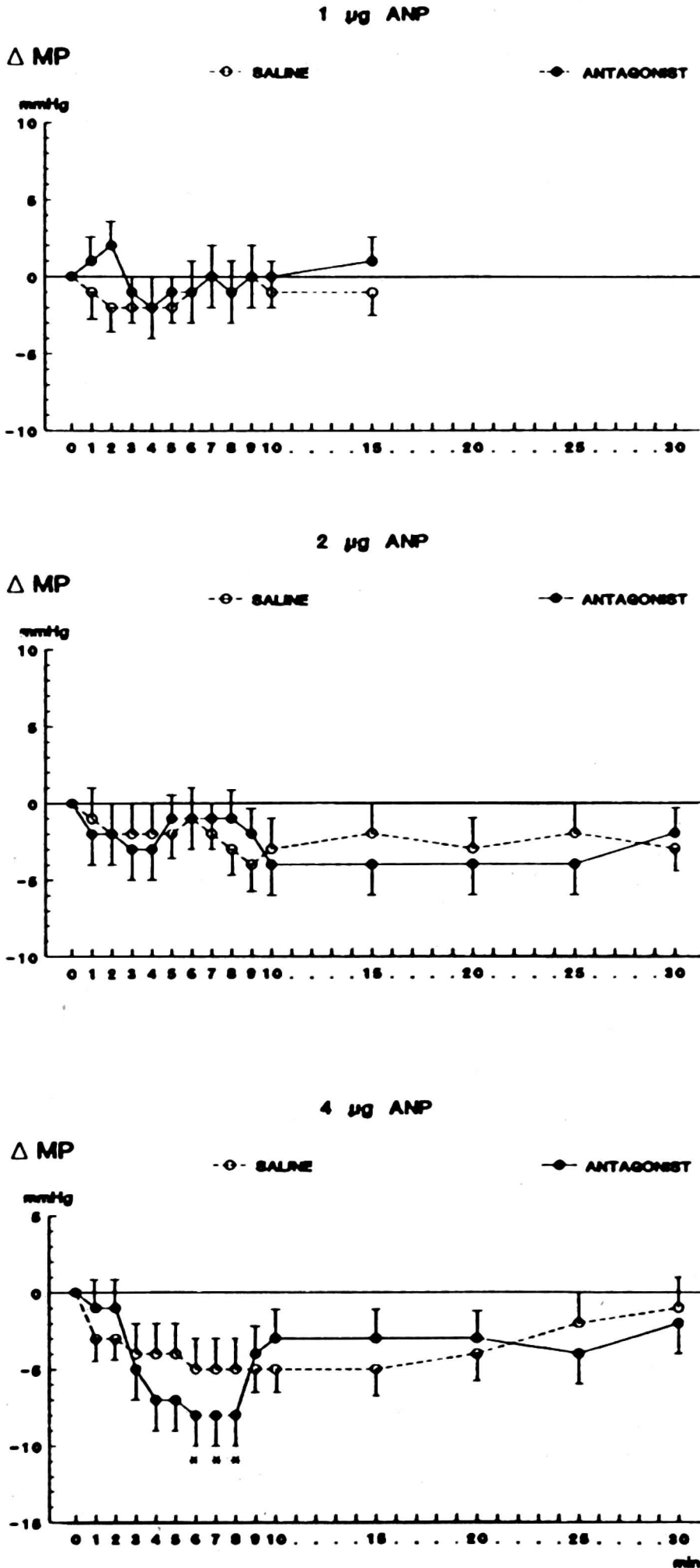


Fig. 5. Changes of mean arterial pressure (Δ HR) after i.v. injection of atrial natriuretic peptide (ANP) during infusion of saline and of V_1 antagonist (dEt₂AVP) spontaneously hypertensive (SHR, n = 10) rats. Asterisks (*) above and below the lines designate significant differences from the preinjection values. Symbols (x) between lines indicate significant differences between saline and V_1 antagonist infusion. Mean \pm SE are shown.

*, x — p < 0.05.

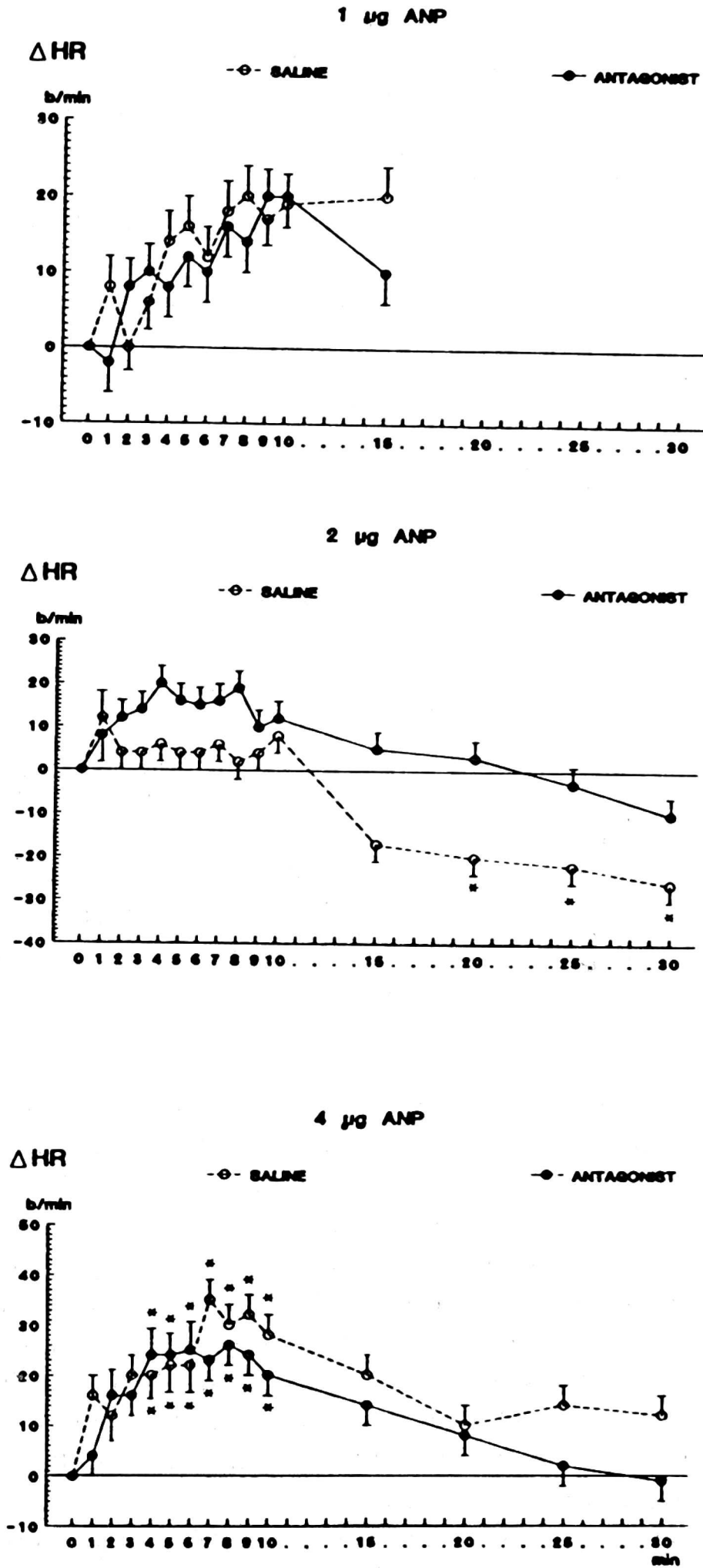


Fig. 6. Changes of heart rate (Δ HR) after i.v. injection of atrial natriuretic peptide (ANP) during infusion of saline and of V_1 antagonist (dEt₂AVP) in spontaneously hypertensive (SHR, n = 10) rats. For symbols and abbreviations see Fig. 5.

In SHR the overall analysis of variance for the time course of HR changes revealed a significant delayed decrease in HR after 2 μg of ANP and a significant increase in HR after injection of 4 μg ANP (Fig. 6). Analysis of HR changes 5 min after injection of ANP (i.e. at the time of greatest SP reduction) disclosed small but significant increase in HR after 1 and 4 μg during saline and after 2 and 4 μg of ANP during dEt₂AVP infusion. No significant differences in HR changes during saline and dEt₂AVP infusion were found in WKY and SHR (Table 1).

Changes of blood pressure and HR after administration of ANP were significantly smaller during infusion of V₁ antagonist than during infusion of vasopressin (Table 1).

DISCUSSION

The present study indicates that elevation of blood vasopressin concentration significantly enhances hypotensive and HR accelerating effects of atrial natriuretic peptide in normotensive and spontaneously hypertensive rats; both effects being better expressed in SHR than in WKY. It is also demonstrated that in both strains blockade of V₁ receptors mediating pressor effects of vasopressin does not interfere with effects of ANP on blood pressure and heart rate under resting conditions.

In SHR ANP tended to be more effective in eliciting blood pressure reduction; the finding which is consistent with other studies (9—11). Intravenous infusion of AVP elicited significant elevation of blood pressure, which was significantly greater in SHR than in WKY; the latter being in agreement with the study of Mohring et al. (12) who demonstrated markedly higher pressor responsiveness of stroke prone SHR to infusion of vasopressin.

Under control conditions doses of ANP used in the present study were close to the threshold hypotensive dose and were not able to cause consistent blood pressure and heart rate responses. This was especially visible in SHR.

On the other hand, injection of ANP during vasopressin infusion elicited consistent significant reductions of blood pressure and accelerations of HR both in WKY and in SHR; the maximum effects being significantly greater in the latter group. In both groups each dose of ANP administered during AVP infusion reduced blood pressure to a level observed during prevasopressin period. Thus, ANP effectively abolished pressor effects of vasopressin infusion.

Taking into account the rate of infusion of AVP in the present study and assuming that its clearance is equal to 3 ml/100 g of the body weight (15) it may be estimated that the blood vasopressin concentration in the present experiments increased by no more than 40 pg/ml and was confined within physiological or pathophysiological range.

Significantly greater hypotensive effectiveness of ANP after elevation of blood vasopressin concentration might have been a result of a higher

vasoconstrictory tone induced by AVP. An alternative explanation could be an interaction between ANP and vasopressin at a cellular level. Existence of a mutual antagonism between ANP and AVP at the level of second messengers in smooth muscles is suggested by the *in vitro* studies. It has been reported that ANP inhibits AVP-stimulated ^{45}Ca uptake and AVP-induced cell contraction in primary cultures of vascular smooth muscles cell (7). In addition, it has been demonstrated that activation of protein kinase C (which mediates AVP action through V_1 receptors) attenuates accumulation of cGMP in smooth muscles cells induced by ANP (8).

In view of the data above demonstrating enhanced responsiveness to ANP after elevation of blood vasopressin concentration as well as other studies reporting increased blood AVP concentration in SHR and increased hypotensive responsiveness to ANP in this strain (9—11, 16—18), it seemed essential to test the hypothesis whether the greater hypotensive effectiveness of ANP may be related to elevated concentrations of endogenous vasopressin in SHR, similarly as it was found during administration of AVP in series 1 of this study.

The results presented in Series 2 of the present study do not support this possibility. Blockade of V_1 receptors which are known to mediate the vasoconstrictory effect of vasopressin did not influence significantly hypotensive effectiveness of ANP neither in WKY nor in SHR. In the latter group administration of $4\ \mu\text{g}$ of ANP resulted in a significant reduction of MP which was absent during saline administration. However, the overall analysis of variance did not disclose significant differences between control and dEt_2AVP periods. The present results indicate that, at least in WKY, the basal levels of endogenous vasopressin do not interfere with hypotensive effects of ANP. With regard to SHR an interaction between ANP and basal levels of vasopressin cannot be entirely excluded since infusion of V_1 antagonist elicited blood pressure decrease and heart increase in this strain. Thus, the effects of ANP might have been limited by preexisting lower vasoconstrictory tone and tachycardia.

With regard to the latter effects, significant reduction of blood pressure after single injection of V_1 antagonists or AVP antibodies was previously observed in SHR by other investigators (16, 18).

In summary, the presents results indicate that elevation of blood vasopressin concentration increases hypotensive action of ANP. Administration of ANP abolishes pressor effects of exogenous vasopressin in WKY and in SHR. On the other hand, basal levels of AVP acting through V_1 receptors probably do not interfere with hypotensive effects of ANP in both strains.

Acknowledgments: The authors wish to thank to Calbiochem-Behring Corp. and to Professor Maurice Manning from the Medical College of Ohio, USA for generous gifts of arginine⁸ vasopressin and dEt_2AVP used in the present study. This study was partly financially supported by the Research Project Cardiovascular Disease CPBR 11.6 Bk/38.

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Received: May 21, 1991

Accepted: November 28, 1991

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