Vol. 146, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1987 Pages 80-86

DECREASED SENSITIVITY OF SPONTANEOUSLY HYPERTENSIVE RAT AORTIC SMOOTH MUSCLE TO VASORELAXATION BY ATRIOPEPTIN III

Marie D. Sauro and David F. Fitzpatrick

Department of Pharmacology and Therapeutics,
University of South Florida, College of Medicine,
Tampa, Florida 33612

Received May 28, 1987

SUMMARY: The effects of a synthetic form of Atrial Natriuretic Factor (ANF) on spontaneously hypertensive rat aortic smooth muscle were investigated using either an alpha-adrenoceptive agonist (phenylephrine) or an agent which partially depolarized the plasma membrane (20mM KCl) as a contractile agent. The relaxant response was studied under conditions resembling normal physiological calcium ion levels (1.5mM) as well as over a range of calcium ion concentrations (0.1-2.5mM). The results demonstrate a hyporesponsiveness of hypertensive aorta to vasorelaxation induced by synthetic ANF, which is more apparent when the tissue is contracted with KCl. The results also suggest that ANF, which has been shown previously to inhibit intracellular and receptor operated calcium channel mobilization only, may additionly work through a mechanism which is related to the voltage induced calcium flux across the membrane, which also is inhibited less in hypertensive smooth muscle.

It is now well documented that mammalian atria produce a peptide hormone which is released into the bloodstream upon atrial distention (1-3). When administered into a conscious animal, this substance causes natriuresis, diuresis, kaliuresis, vasodilatation, and inhibition of renin and aldosterone secretion, hence the name, Atrial Natriuretic Factor (ANF) (4-7). It is becoming increasingly evident that ANF plays an important role in fluid balance and blood pressure regulation. However, the role, if any, of ANF in the development of hypertension is yet to be established.

Hypertension may be described in terms of a disruption in any of the mechanisms which are involved in fluid homeostasis. In the hypertensive state, in which pathogenic mechanisms are not well understood, there may be an altered response of hypertensive aortic smooth muscle to the vasorelaxant effects of ANF. It is the intent of this study to investigate the possibility of a pathologic vascular response to ANF-induced vasodilatation using the spontaneously hypertensive rat, the most thoroughly studied model of genetic hypertension.

0006-291X/87 $1.50

METHODS

Tissue preparation

Male, 8-12 week old, age matched control Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) were used in this study. One day prior to experimentation, systolic blood pressure was taken using the tail cuff method (Narco-biosystems PE-300 Programmed Electro-Sphygmomanometer).

Rats were anesthetized with sodium pentobarbital (50mg/kg) and bled. Thoracic aortae were excised, bathed in Krebs buffer (mM): (0.027 EDTA, 136 NaCl, 5.6 KCl, 20 $NaHCO_3$, 1.2 $MgSO_4 \cdot 7H_2O$, 1.2 NaH_2PO_4, dextrose 1.0g/L) containing 1.5mM calcium chloride and aerated with 95% oxygen, 5% carbon dioxide. After clearing extraneous tissue, helical strips (approximately 3-4 mm by 12-17 mm) were cut and mounted in tissue chambers containing Krebs solution at 37°C. Strips were attached to Grass FT03C force displacement transducers and left to equilibrate for 2 hours with 1500mg of preload which allowed for maximal contractile response when treated with agonist. The solution in each bath was changed every 15 minutes with Krebs warmed at 37°C.

Pharmacodynamic studies

In each set of experiments following the equilibration period, tissues were rinsed in Krebs buffer without calcium chloride. Due to the loss of spontaneous myogenic tone in SHR strips (8), preload was readjusted to match that of WKYs.

In the first set of experiments, strips were contracted by adding 1.5mM calcium chloride and either phenylephrine (10^{-7}M) or 20mM KCl (Krebs solution was modified in order to maintain osmolarity). After producing a sustained contraction, a synthetic form of ANF, Atriopeptin III (AIII), was added in cumulative concentrations of 10^{-10}M to 10^{-8} M. Relaxations were calculated as percent maximal relaxation induced by AIII. For the second set of experiments, strips incubating in zero-calcium were treated with either phenylephrine (10^{-5} M) or KCl (20mM) and then contracted with calcium chloride in cumulative concentrations of 0.1 to 2.5mM. Contractions were calculated as percent maximum grams tension produced by calcium chloride. Lastly, WKY and SHR strips were contracted with 1.5mM calcium chloride and phenylephrine in cumulative concentrations of 10^{-9}M to 10^{-6}M in the presence or absence of AIII. Contractions were calculated as percent maximal grams tension produced by phenylephrine. IC_{50} values were calculated on a log scale according to the method of Fleming, et al. (9). In each set of experiments, statistical significance was established using the Student's t-test.

RESULTS

Figure 1 illustrates the relaxant response of WKY and SHR aorta to cumulative concentrations of AIII following (a) KCl or (b) phenylephrine induced contractions. In both instances, there was a significant difference in responsiveness between normotensive and hypertensive aorta. When using KCl as a contractile agent, this difference between SHR and WKY was quite evident with a significant difference ($p<.002$) in IC_{50} values (WKY:2.84×10^{-10} M, SHR:7.70×10^{-10} M). Although the shift in the IC_{50} value was not as dramatic when phenylephrine was used (WKY:1.62×10^{-9}M, SHR:2.72×10^{-9} M), the difference was found to be statistically significant at lower AIII concentrations.

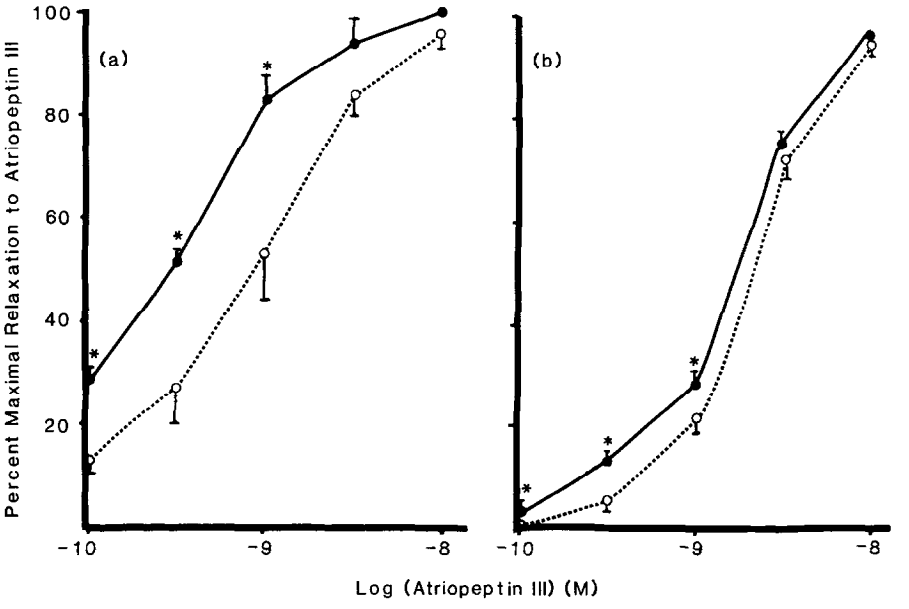

Figure 1. Dose-response curves for Atriopeptin III induced relaxation of -●- WKY and -○- SHR aortic strips precontracted with (a) 20 mM KCl or (b) 10^{-7} M phenylephrine. Values are expressed as means ± SEM. Asterisks denote statistical significance.

Figures 2 and 3 are calcium dose-response curves using either phenylephrine (fig. 2) or KCl (fig. 3) as the contractile agent. Although pretreatment with low AIII concentrations had no statistically

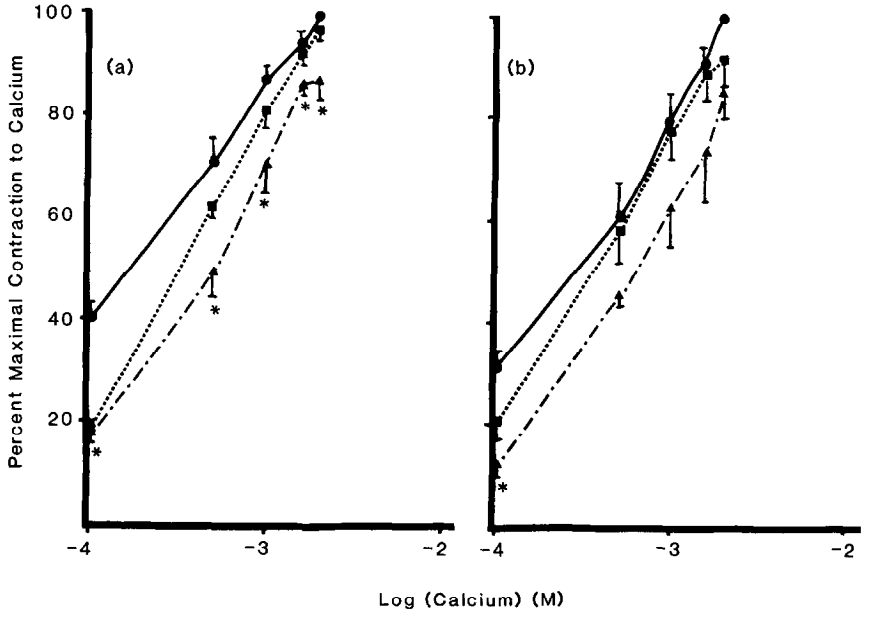

Figure 2. $CaCl_2$ dose response curves for (a) WKY and (b) SHR aortic strips using phenylephrine in the absence or presence of various concentrations of AIII. (Control -●-; 10^{-10} M ---■---; 10^{-9} M --▲--). Values are expressed as means ± SEM. Asterisks denote statistical significance.

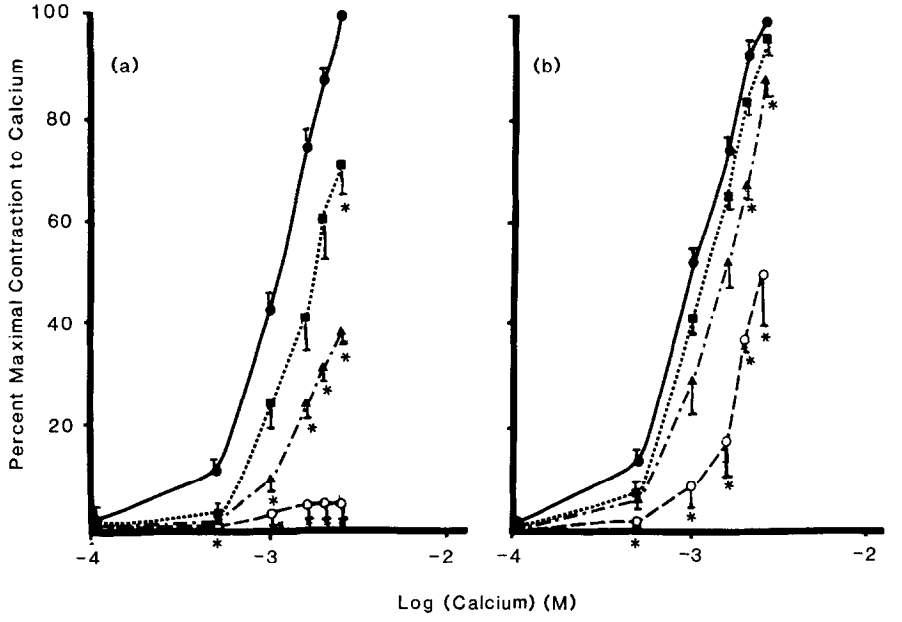

Figure 3. $CaCl_2$ dose response curves for (a) WKY and (b) SHR aortic strips using KCl in the absence or presence of various concentrations of AIII. (Control -●-; 10^{-10} M ---■---; 10^{-9} M ---▲---; 10^{-8} M -○-) Values are expressed as means ± SEM. Asterisks denote statistical significance.

significant effect on contractile response, there was a difference in the IC_{50} values which was greater for WKY than for SHR aorta, when either phenylephrine or KCl was used. When using higher AIII concentrations, however, there was a significant shift of IC_{50} values ($p<.05$) for WKY aorta, but relatively little effect on SHR aorta. This shift was again, more pronounce when the tissue was partially depolarized.

In figure 3, when KCl contracted tissues were pretreated with 10^{-8} M AIII, there was approximately 95% inhibition of contractile response for WKY aorta compared to approximately 50% inhibition for SHR aorta.

Figure 4 illustrates the results obtained by contracting strips with cumulative concentrations of phenylephrine in the absence or presence of AIII. Pretreatment with low concentrations of AIII was not very effective in producing relaxation, but once again, WKY aorta showed greater relaxation to AIII than SHR. After treatment with higher concentrations of AIII, there was a 6-fold shift to the right of the IC_{50} value for WKY aorta relative to a 1.5-fold shift for SHR aorta.

DISCUSSION

The results of this study demonstrate a hyporesponsiveness of hypertensive rat aortic smooth muscle to vasorelaxation by Atriopeptin III. This could be seen when using either an alpha

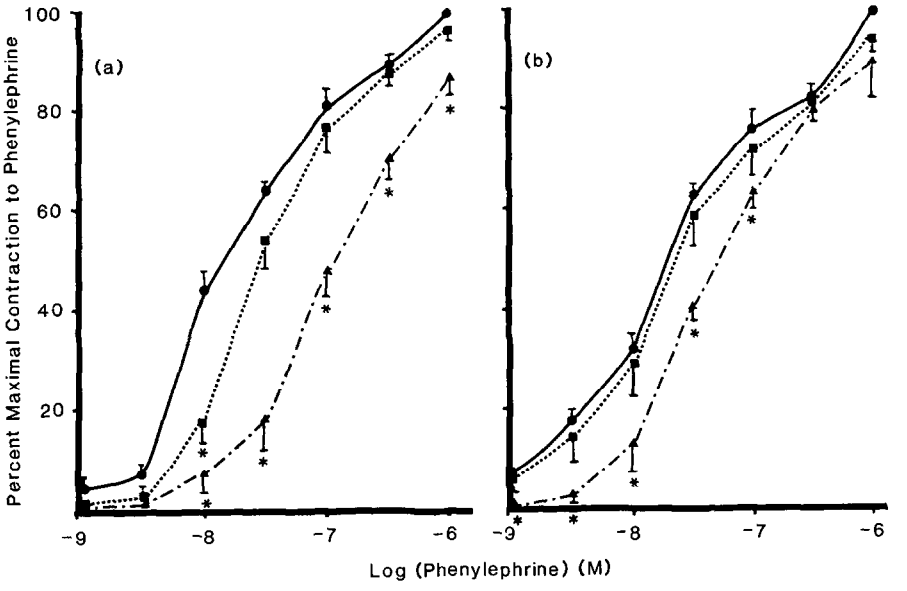

Figure 4. Dose-response curve for phenylephrine-induced contraction of (a) WKY and (b) SHR aortic strips in the absence or presence of various concentrations of AIII. (Control —●—; 10^{-10} M --■--; 10^{-9} M ---▲---) Values are expressed as means ± SEM. Asterisks denote statistical significance.

adrenoreceptor agonist or an agent which causes partial depolarization to elicit a contractile response. Hyporesponsiveness of SHR aorta is more apparent when KCl rather than when phenylephrine is used as the contractile agent.

It is thought that alpha adrenoceptive agents mobilize calcium by more than one mechanism. Extracellular calcium may enter through receptor operated calcium channels (ROC) when the appropriate signal is given. Also as a consequence of receptor stimulation, phosphoinositide metabolism leads to the production of inositol 1,4,5-trisphosphate (IP_3) which may be the signal for release of intracellular calcium stores from the sarcoplasmic reticulum (10). Depolarizing agents such as KCl, however, stimulate the opening of potential- or voltage-operated channels (VOC), but have no known direct effect on intracellular calcium mobilization. It is possible that when tissue is contracted with agonist, it is more resistant to relaxation than if contracted with KCl since both intra- and extra-cellular sources of calcium are utilized. When both sources are mobilized in hypertensive tissue, then atrial peptides will not be as effective in inducing relaxation. This may suggest that atrial peptides, which have been shown to inhibit intracellular and ROC calcium mobilization (11), may additionally operate through a mechanism which is related to potential-induced calcium flux across the plasma membrane and which is inhibited less in hypertensive vascular smooth muscle. ANF has previously been shown to be effective

in inhibiting contractions induced by depolarization with low (12), but not high (13) levels of KCl. This may be of some significance since lower levels of depolarization are more likely to occur in vivo. It should also be noted that tissues contracted with an extremely high concentration of agonist are also less likely to respond to ANF.

Altered responsiveness to atrial peptides is also seen in other models of hypertension. In mineralocorticoid (DOCA)-induced hypertension, it was found that the rate of relaxation produced by ANF was slower for hypertensive aorta than for normotensive when precontracted with norepinephrine (14). Using rats with renal hypertension (1 kidney, 1 clip), a significantly lower number of ANF binding sites were found in hypertensive rat aorta, and in addition a decreased sensitivity of hypertensive rat aorta to ANF-induced relaxation (15). Winquist found a decreased sensitivity of SHR aorta to ANF as well as to other vasodilators (16).

In each model, there is suggestion of an impaired ability of vascular smooth muscle to respond to vasodilation by atrial peptides. It has been reported that the vasculature from hypertensive animals develops an altered sensitivity to various vasoactive substances. The mechanisms involved in the altered responsiveness have been classified by Winquist into two main areas: drug-receptor interaction and altered membrane permeability to monovalent and divalent cations which may increase vascular tone (17). It has been shown that circulating levels of ANF in SHRs are actually higher than control (18). This would further suggest that the defect is not so much in the synthesis, release or amount of ANF in the circulation, but rather in the vascular response specifically in the cascade of events which is calcium mediated. Comparing vasorelaxation in the normotensive and hypertensive states may give some insight into the differences that exist between the two and could suggest that hyporesponsiveness to ANF may play a role in the development of high blood pressure.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the suggestions of Dr. Joseph J. Krzanowski and secretarial assistance of Ms. Jill Culbreth in the preparation of this manuscript.

This study was supported by Biomedical Research Support Grant 507 RR05749, Division of Research Resources, National Institute of Health.

REFERENCES

1. Currie, M.G., Geller, D.M., Cole, B.R., and Needleman, P. (1984) Proc. Natl. Acad. Sci. USA 81, 1230-1233.

2. Sagnella, G.A. and MacGregor, G.A. (1984) Nature 306, 666-667.
3. Dietz, J.R. (1984) Amer. J. Physiol. 247, R1093-R1096.
4. deBold, A.J. Borenstein, H.B., Veress, A.T., and Sonnenberg, H. (1981) Life Sci. 28, 89-91.
5. deBold, A.J. (1982) Proc. Soc. Exp. Biol. Med. 170, 133.
6. Currie, M.G., Geller, D.M., Cole, B.R. (1983) Science 221, 71-73.
7. Garcia, R., Thibault, G., Cantin, M., Genest, J. (1984) Amer. J. Physiol. 247, R34-R39.
8. Fitzpatrick, D.F. and Szentivanyi, A. (1980) Clin. and Exp. Hypertension 2, 1023-1037.
9. Fleming, W.W., Westfall, D.P., De LaLande, I.S. and Jellette, L.B. (1972) J. Pharm. and Exp. Ther. 181, 339-345.
10. Abdel-Latif, A.A. (1986) Pharm. Rev (ASPET) 36, 228-257.
11. Meisheri, K.D. and Taylor, C.J. (1986) Amer. J. Physiol. 250, C171-C174.
12. Kleinert, H.D., Maack, T., Atlas, S.A., Januszewicz, A., Sealey, J.E. and Laragh, J.H. (1984) Hypertension 6, I-143-I147.
13. Taylor, C.J. and Meisheri, K.D. (1986) J. Pharm. and Exp. Ther. 237, 803-808.
14. Thompson, L.P. and Webb, R.C. (1986) Hypertension 8, I-146-I150.
15. Schiffrin, E.L., St. Louis, J., Garcia, R., Thibault, G., Cantin, M. and Genest, J. (1986) Hypertension 8, I-141-I145.
16. Winquist, R.J., Faison, E.P., Baskin, E.P., Bunting, P. B., Nutt, R.F, and Callahan, L.T. (1984) J. Hypertension 2, 325-327.
17. Winquist, R.J., Webb, R.C., and Bohr, D.F. (1982) Fed. Proc. 41, 2387-2393.
18. Gutkowska, J., Horky, K., Lachance, C., Racz, K., Garcia, R., Thibault, G., Kuchel, O., Genest, J., and Cantin, M. (1986) Hypertension 8, I-137-I140.