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RAPID COMMUNICATION

DEFECTIVE CYCLIC GMP ACCUMULATION IN SPONTANEOUSLY HYPERTENSIVE RAT AORTA IN RESPONSE TO ATRIAL NATRIURETIC FACTOR

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It has been demonstrated in our laboratory (1) and others (2) that aortic smooth muscle from spontaneously hypertensive rats is hyporesponsive to vasodilatation by a synthetic analogue of atrial natriuretic factor (ANF). Although its mechanism of action has not been elucidated, it is known that ANF stimulates cyclic guanosine 3',5'-monophosphate (cGMP) production in a number of tissues including vascular smooth muscle (3,4). It was first thought that cGMP was involved in the contractile response of certain types of smooth muscle (5), but more recently it has been established that nitrovasodilators and other vasorelaxant substances stimulate guanylate cyclase and increase cGMP production (6,7). Also, lipophilic derivatives of cGMP as well as cAMP relax various smooth muscle preparations (8). cGMP activates cGMP-dependent protein kinase and it has been suggested that this somehow mediates the dephosphorylation of myosin light chain, ultimately causing relaxation of smooth muscle (9,10). cGMP also decreases the availability of cytosolic Ca^{++} needed for contraction, possibly through enhancement of either the sarcolemmal extrusion Ca^{++} ATPase (11) or sequestration into the sarcoplasmic reticulum (12), or both.

It can then be postulated that a defect in any one or more of these steps could lead to a pathogenic vascular response and, therefore, altered responsiveness to vasoactive substances. This paper is the first report of an attempt to examine the biochemical mechanisms responsible for altered vasorelaxant response to ANF in the genetic model of hypertension and is consistent with previous findings (1) of decreased responsiveness of SHR aorta to ANF.

MATERIALS AND METHODS

One day prior to experimentation, systolic blood pressures of 8- to 9-week-old, age matched Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) (Charles River, Wilmington, MA) were taken using the tail cuff method (Narco-biosystems PE-300 Programmed Electro-Sphygmomanometer). Systolic blood pressures differed significantly ($P < 0.001$) (WKY: 108.7 ± 8.3 mm Hg, SHR: 141.8 ± 6.3 mm Hg) but weights did not (WKY: 171.3 ± 15.8 g, SHR: 182.8 ± 15.0).

Rats were first anesthetized with sodium pentobarbital (50 mg/kg) and then killed by an injection of saturated KCl directly into the heart. Thoracic aortae were removed, cleared of fascia, and bathed in aerated (95% O_2/5% CO_2) Krebs buffer (0.027 mM EDTA,

136 mM NaCl, 5.6 mM KCl, 20 mM $NaHCO_3$ 1.2 mM $MgSO_4 \cdot 7H_2O$, 1.2 mM NaH_2PO_4 and 1.0 g/l dextrose) containing 1.5 mM calcium chloride. After helical strips were cut, they were divided into pieces (2-2.5 mm by 6-8 mm) and were left to equilibrate in aerated Krebs buffer maintained at 37° plus calcium for 1.5 hr. Krebs solution was changed every 20 min. Following equilibration, individual pieces were treated with 10^{-7} M phenylephrine (Sigma) for 3 min and then with synthetic ANF, Atriopeptin III (AIII) (Calbiochem-Behring Diagnostics) for 15 min at which time the reaction was terminated with ice-cold 12% trichloroacetic acid (TCA).

In preliminary experiments tissues were frozen and thawed 3 times. The insoluble material was then homogenized with 12% TCA and centrifuged. The cyclic nucleotide content of the second extract was negligible relative to the first extract from the frozen and thawed tissue. This established that virtually all of the cyclic nucleotides were extracted from the small section of aorta by this freeze/thaw method, which was used in subsequent experiments. Preliminary data also showed that a 15 min AIII treatment produced maximal cyclic nucleotide production and also correlated with the time required to produce a plateau relaxation response in isolated aortic smooth muscle strips precontracted with agonist.

TCA extracts were then pipetted into columns (0.7 cm i.d.) containing 0.25 g of neutral Alumina (Merck Sharp & Dohme), which was packed by gravity sedimentation through glass-distilled water. Columns were washed successively with 5 ml of 0.5 M perchloric acid, 5 ml of water and 0.2 ml of 0.2 M sodium acetate, pH 6.2. Cyclic nucleotides were then eluted with 1.0 ml of 0.2 M sodium acetate, pH 6.2, with a recovery of 85 ± 5%. This procedure accomplishes the neutralization of the sample and removes several impurities present in both tissue and medium which interfere with the radioimmunoassay. Aliquots of samples were acetylated and assayed for cGMP and cAMP by radioimmunoassay (13) using rabbit polyclonal antibodies and ^{125}I-tyrosine methyl esters of 2'-O'-succinyl cyclic nucleotides prepared in our laboratory (14).

Tissues were dried to a constant weight, and cyclic nucleotide data are expressed as femtomoles (fmol) or picomoles (pmol) per mg dry weight. Statistical significance was determined using a two-tailed Student's *t*-test for unpaired data.

RESULTS AND DISCUSSION

Table 1 confirms the finding that cAMP levels in aortic smooth muscle are not affected significantly by ANF (15). No differences were found when comparing cAMP levels in aortas of WKY and SHR.

Table 1. Effects of Atriopeptin III on cAMP levels

[AIII] (M)	cAMP (pmol/mg tissue) WKY	N	SHR
Basal	4.4 ± 1.5	5	4.3 ± 1.4
10^{-9}	2.0 ± 1.1	3	2.2 ± 0.7
10^{-8}	5.7 ± 2.4	3	4.7 ± 2.1
3×10^{-8}	5.6 ± 4.1	3	3.2 ± 2.0
10^{-7}	5.8 ± 2.7	4	5.8 ± 2.4
3×10^{-7}	6.1 ± 2.8	4	4.8 ± 2.0

Values represent the mean pmol cAMP/mg tissue ± SEM from 3 - 5 experiments. N = number of experiments.

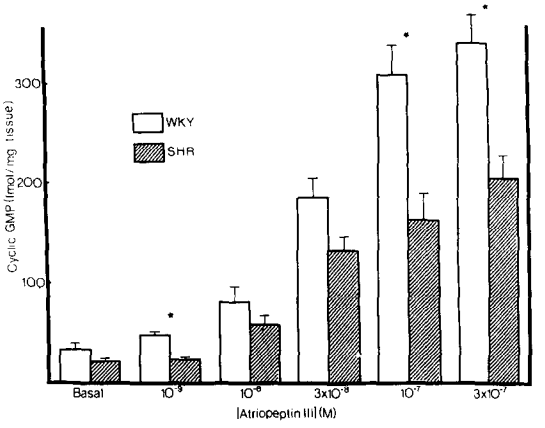

Fig. 1 Effects of Atriopeptin III on cGMP accumulation in WKY versus SHR aortic smooth muscle. Data are expressed as the mean value of fmol cGMP produced/mg tissue ± SEM from 5 experiments. Asterisks denote a statistically significant difference in cGMP levels between WKY and SHR (P < 0.05).

Figure 1 illustrates the magnitude and variation of AIII-stimulated cGMP accumulation in SHR and WKY aortae. Although basal levels were not found to be significantly different (WKY: 32.2 ± 5.7 fmol/mg tissue; SHR: 21.0 ± 1.8 fmol/mg tissue), the difference in stimulated cGMP accumulation between the two was found to be statistically significant at both low and high AIII concentrations.

The regulation of cyclic nucleotide levels within the cell is dependent upon rates of synthesis and degradation. It has been reported that there are no differences in cGMP phosphodiesterase activity between hypertensive and normotensive rat aorta (16), suggesting that the defect may reside in the synthesis of cGMP or its activation of cGMP-dependent protein kinase. Also, a plasma membrane bound form of guanylate cyclase has been found to be intimately linked to the ANF receptor (17,18). Since ANF directly activates particulate guanylate cyclase, there may be a decrement in that specific enzyme activity in the hypertensive state.

The attenuated cGMP response in SHR aorta may have important implications. Firstly, less cGMP is available to activate cGMP-dependent protein kinase which as previously mentioned is involved in the cascade of events resulting in vasorelaxation. Secondly, less cGMP is available to exert its effects on intracellular Ca^{++} mobilization (11,12). This would presumably result in higher Ca^{++} levels available to complex with calmodulin and cause vasoconstriction in SHR smooth muscle. Both of these pathways could explain the hyporesponsiveness of SHR aortae to AIII.

This report is the first which compares cyclic nucleotide levels in spontaneously hypertensive versus normotensive rat vascular smooth muscle. Marsh et al. (19) have reported a significant increase in urinary cGMP excretion with infusion of synthetic ANF in WKY, but only a slight increase in SHR. Since a variety of tissues respond to ANF with increased cGMP production, this may indicate a defect in cGMP accumulation in response to ANF in other target tissues as well as the vasculature.

There are still many components and complexities in the various second messenger pathways involved in smooth muscle relaxation that require further examination. This report demonstrates one biochemical defect that is consistent with the notion of hypo-

responsiveness of vascular smooth muscle from hypertensive rats to vasorelaxation by ANF. Whether other enzymes of tissues from hypertensive rats respond abnormally to ANF remains to be tested, and such studies may aid in understanding the mechanism of action of this specific vasoactive hormone.

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Addendum While this manuscript was in preparation, Otsuka et al. (20) reported that ANF-stimulated cGMP levels in rat aorta were attenuated in DOCA and coarctation models of hypertension.

REFERENCES

1. M.D. Sauro and D.F. Fitzpatrick, Biochem. biophys. Res. Commun. 146, 80 (1987).
2. R.J. Winquist, E.P. Faison, E.P. Baskin, P.B. Bunting, R.F. Nutt and L.T. Callahan, J. Hypertension 2, 325 (1984).
3. S.A. Waldman, R.M. Rapoport and F. Murad, J. biol. Chem. 259, 14332 (1984).
4. R.J. Winquist, E.P. Faison, S.A. Waldman, F. Schwartz, F. Murad and R.M. Rapoport, Proc. natnl. Acad. Sci. U.S.A. 81, 7661 (1984).
5. E.W. Dunham, M.K. Haddox and N. Goldberg, Proc. natnl. Acad. Sci. U.S.A. 71, 815 (1974).
6. H. Kimura, C.K. Mittal and F. Murad, Nature, Lond. 257, 700 (1975).
7. S. Katsuki, W. Arnold, C. Mittal and F. Murad, J. Cyclic Nucleotide. Res. 3, 23 (1977).
8. R.M. Rapoport, M.B. Drazin and F. Murad, Proc. natnl. Acad. Sci. U.S.A. 79, 6470 (1982).
9. R. Fiscus, R.M. Rapoport and F. Murad, J. Cyclic Nucleotide Protein Phosphorylat. Res. 9, 415 (1983).
10. F. Murad, R.M. Rapoport and R. Fiscus, J. cardiovasc. Pharmac. 7, S111 (1985).
11. E. Suematsu, M. Hirata and H. Kuriyama, Biochim. biophys. Acta 773, 83 (1984).
12. C.H.C. Twort and C. Van Breemen, Clin. Sci. 74, 58p (1988).
13. G. Brooker, J.F. Harper, W.L. Terasaki and R.D. Moylan, Adv. Cyclic Nucleotide Res. 10, 1 (1979).
14. R.G. Coffey and J. Hadden, Cancer Res. 43, 150 (1983).
15. R.R. Fiscus, R.M. Rapoport, S.A. Waldman and F. Murad, Biochim. biophys. Acta 846, 179 (1985).
16. R.V. Sharma and R.C. Bhalla, Biochim. biophys. Acta 526, 479 (1978).
17. T. Kuno, J.W. Andresen, Y. Kamisaski, S.A. Waldman, L.Y. Chang, S. Saheki, D.C. Leitman, M. Nakone and F. Murad, J. biol. Chem. 261, 5817 (1986).
18. A.K. Paul, R.B. Marala, R.K. Jaiswal and R.K. Sharma, Science 235, 1224 (1987).
19. E.M. Marsh, A.A. Seymour, A.B. Haley, M.A. Whinnery, M.A. Napier, R.F. Nutt and E.H. Blaine, Hypertension 7, 386 (1985).
20. Y. Otsuka, A. DiPiero, E. Hirt, B. Brennaman and W. Lockette, Am. J. Physiol. 254, H163 (1988).