PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **184**, 123–126 (1987)

RAPID COMMUNICATIONS

ACUTE STRESS INCREASES PLASMA CONCENTRATIONS OF ATRIAL NATRIURETIC PEPTIDES

DAVID A. BLIZARD AND MARIANA MORRIS

Department of Physiology and Pharmacology,
Bowman Gray School of Medicine of Wake Forest University,
Winston-Salem, North Carolina 27103

5 min exposure of inbred Maudsley Reactive male rats to intermittent foot-shock resulted in an approximate doubling of plasma atrial natriuretic peptides ANP (Control grp mean = 62.12 ± 8.74; Stressed grp mean = 128.70 ± 26.63 pg/ml) and 25 min exposure resulted in a three-fold increase (Stressed grp mean = 187.88 ± 39.24 pg/ml). In the second experiment exposure of genetically heterogeneous Wistar male rats to 15 min of intermittent foot-shock produced a 10-fold increase in plasma ANP (Control grp mean = 45.76 ± 6.05; Stressed grp mean = 471.20 ± 58.49 pg/ml). The magnitude of the increase in plasma ANP produced by acute stress is as large as the increase caused by volume expansion and administration of various pharmacological agents and therefore delineation of biological role of ANP must take account of its potential role as a stress-hormone.

The release of atrial natriuretic peptides (ANP) is stimulated by a variety of factors including acute volume expansion (1,2), hypertonic saline (2,3), vasopressin administration (4) as well as bolus injections of angiotensin II and phenylephrine (5). It is commonly believed that it is the pressor effects of these treatments which leads to heightened release of ANP by increasing atrial distension (6). In addition to the above, a variety of stressors are known to produce acute increases in blood-pressure and , as noted above, might be expected to increase release of ANP. This possibility was explored in the following experiments. It was predicted that the application of a commonly used laboratory stressor would result in elevation of plasma ANP.

Materials and Methods. In the first experiment, adult male Maudsley Reactive (MR/Har) male rats (7) were exposed to intermittent foot-shock (duration 1 sec; intensity, 3 ma.) at 15 second intervals for 5, 15 and 25 minutes and sacrificed by decapitation immediately after termination of the stressor. Controls were sacrificed during the same time period as the stressed rats approximately halfway through the light phase of a 15 hrs light/9 hrs dark diurnal cycle. The experiment was divided into two phases which were carried out one month apart with control and experimental animals included in each phase. Whole blood was collected in heparinized tubes on ice and plasma stored at -70°C until the assay for ANP was carried out.

Biochemical procedures: plasma was extracted with a C-18 Sep-Pak cartridge (Waters Instruments) which had been pre-treated with methanol, water and 0.1% BSA in phosphate buffered saline. The Sep-Pak was washed with water and the ANP eluted with a solution on acetonitrile (75%) and 4.0% acetic acid (25%).

0037-9727/87 $1.50

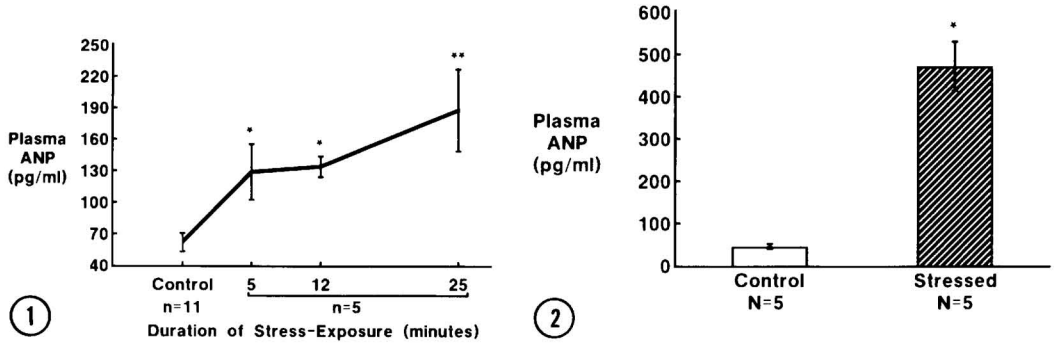

Figure 1. Plasma atrial natriuretic response (ANP) response to intermittent foot-shock in Maudsley Reactive male rats. (* p <0.06, ** p <0.004 vs control by post-hoc test.)

Figure 2. Plasma atrial natriuretic peptide (ANP) response to 15 min intermittent foot-shock in Wistar male rats. (* p <0.001 vs control by analysis of variance)

ANP was measured using a specific and sensitive radioimmunoassay (2). The rat anti-serum used in this study was generated against synthetic rat ANP coupled to bovine thyroglobulin with carbodiimide and genrously supplied by Dr. R.L. Eskay. The synthetic standard, atriopeptin III, was purchased from Peninsula. The assay volume was 400 µl with a 24-hr incubation period. A second antibody was used for separation of bound hormone. The mean ED_{90} and ED_{50} for 12 RIAs was 6.2 ± 0.8 and 100.7 ± 12.0 pg.

Results. The results are shown in Figure 1. Exposure to foot-shock resulted in a substantial increase in plasma ANP in MR/Har male rats ($F = 7.87$, 3 and 22, $p < 0.001$) The magnitude of the changes in ANP seen after 5 and 12 minutes of stress represent an approximate doubling of ANP levels compared to control while the increase seen after 25 min of stress represents a three-fold increase compared to control levels. The reliability of the results is supported by the fact that two phases of the experiment which were conducted one month apart (see above) produced essentially the same pattern of results.

Experiment 2. Maudsley Reactive rats are the results of genetic selection for increased behavioral reactivity to mild stress and have been inbred for some 60 generations of brother/sister matings (7). It is therefore conceivable that the results we saw in the experiment described above could reflect the idiosyncratic response of this particular rat strain rather than the response of more widely used rat stocks. To examine this possibility we studied the response of outbred Wistar male rats obtained from the Charles River Co. In the second experiment, guided by the time-course of ANP response to stress observed in the first experiment, we elected to look at ANP levels after 15 min of stress-exposure. Apart from this, the experimental protocol was exactly as described for the first experiment (see above).

The results are shown in Figure 2 where it can be seen that after 15 min exposure to intermittent foot-shock plasma ANP levels in Wistar males were approximately 10-fold the levels seen in controls ($t = 7.22$, df 8, $p = <0.001$). Basal ANP levels did not differ significantly between Wistar and Maudsley rats but the magnitude of

the stress-induced increase was greater in Wistars (Peak ANP levels during stress, $p < .004$).

Discussion. The hypothesis that acute stress will produce increases in plasma ANP is confirmed by the results of both experiments. Exposure to foot-shock produced a three-fold increase in plasma ANP in Maudsley Reactive rats after 25 min of stress and a 10-fold increase in outbred Wistar rats, after 15 min of stress. An earlier experiment has shown that exposure to immobilization stress for 4 hours produced a significant increase in plasma ANP (8) but the results of the present studies indicate that the effects of stress are much more immediate and can be seen within a short time of stress-initiation. Although ANP levels continued to increase through 25 min of stress the scope of the present experiments need to be extended to fully explore the time of peak responsivity.

The fact that the stress-induced increment in plasma ANP was greater in Wistar than in Maudsley rats hints at the possibility of genetic variation in stress-induced ANP-release. However, since the present studies involve comparisons of outbred, (Wistar) with inbred (Maudsley Reactive) rats conducted separately, it would be useful to examine this possibility more closely by carrying out controlled genetic comparisons within the same experiment.

The effects of stress on plasma ANP, as revealed by the present experiments, suggest that it is as potent a stimulator of ANP release as volume loading (1,2) and administration of certain anesthetics (8). Thus, the effects of stress will need to be carefully considered in the characterization and elaboration of the role of these hormones (9). The methodological significance of the present findings should also be considered. If other stressors are found to increase plasma ANP then it will become especially important to minimize the effects of stress when carrying out experimental manipulations. Indeed, the present findings suggest it would be wise to exercise such precautions without more ado.

How stress stimulates the increase in plasma ANP is not addressed by the present studies but the pressor effects of foot-shock are well established and are probably associated with increased venous return and, a likely mediator of ANP-release, increased atrial distension. Alternatively, a recent study (2) has demonstrated that nervous system or pituitary activation may play a role in ANP-release by volume expansion and it is conceivable that either of these two factors could also mediate the effects of stress on ANP.

More generally, the endocrine profile traditionally associated with acute stress must clearly be expanded to include an important set of hormones with natriuretic, diuretic and vasorelaxant properties (9,10). This is an exciting prospect which should lead to both conceptual and empirical advances in stress research as the role of these hormones as potential mediators of stress response is considered.

Acknowledgements. We thank Stefanie Weigerink for technical assistance. Supported by grants MH 39210 to DAB and HL 30845 to MM. Her work was done during the tenure of an Established Investigatorship from the American Heart Association with funds contributed in part by the NC affiliate.

References

1. Lang RE, Tholken H, Ganten D, Luft FC, Ruskoaho H, Unger T. Atrial natriuretic factor- a circulating hormone stimulated by volume loading. Nature 314:264-266, 1985.

2. Eskay R, Zukowska-Grojec Z, Haass M, Dave JR, Zamir N. Characterization of circulating atrial natriuretic peptide in conscious rats: regulation of

release by multiple factors. Science 232:636-639, 1986.

3. Morris M, Rauch A. Specific response of plasma ANF to a hypertonic NaCl stimulus. Soc. Neuroscience in press, 1986.

4. Manning PT, Schwartz D, Katsube NC, Holmberg SW, Needleman P. Vasopressin-stimulated release of atriopeptin: endocrine antagonists in fluid homeostasis. Science 229:395-397, 1985.

5. Katsube N, Schwartz D, Needleman P. Release of atriopeptin in the rat by vasoconstrictors or water immersion correlates with changes in right atrial pressure. Biochem Biophys Res Commun 133:937-944, 1985.

6. Dietz JR. Release of natriuretic factor from the heart-lung preparation by atrial distension. Am J Physiol 247:R1093-1096, 1984.

7. Blizard DA. The Maudsley Reactive and Non-Reactive strains: A North American perspective. Behav Genet 11:469-489, 1981.

8. Horky K, Gutkowska J, Garcia R, Thibault G, Genest J, Cantin M. Effect of different anesthetics on immunoreactive atrial natriuretic factor concentrations in rat plasma. Biochem Biophys Res Comm 129:651-657, 1985.

9. DeBold A. Atrial Natriuretic Factor: A Hormone produced by the heart Science, 230:767-770, 1985.

10. Buckalew VMJ Jr., Hamilton RW, Morris M. Atrial Natriuretic Factor, Adv Int Med 32:in press, 1986.

Received September 3, 1986.
P.S.E.B.M. 1987, Vol. 184.
Accepted October 13, 1986.