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ALTERATIONS IN ATRIAL AND PLASMA ATRIAL NATRIURETIC FACTOR (ANF) CONTENT DURING DEVELOPMENT OF HYPOXIA-INDUCED PULMONARY HYPERTENSION IN THE RAT

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Abstract. Distension of the atrial wall has been proposed as a signal for the increased release of atrial natriuretic factor (ANF) from atrial myocytes in response to perceived volume overload. To determine whether pressure changes resulting from hypertension in the pulmonary circulation may stimulate release of ANF, rats were exposed to chronic hypobaric hypoxia for 3 or 21 days and the ANF concentration in the atria and plasma were determined by specific radioimmunoassay. Exposure to chronic hypoxia resulted in significant increases in hematocrit at both 3 ($p < 0.025$) and 21 days ($p < 0.005$) and in the development of right ventricular hypertrophy (RVH) expressed as the ratio of the weight of the right ventricle to the weight of the left ventricle and septum (RV/LV+S) at both 3 (RV/LV+S = 0.278±0.005) and 21 days (RV/LV+S = 0.536±0.021). After 21 days, left atrial (LA) ANF content was significantly increased in hypoxic rats compared to controls (508±70 ng/mg tissue vs 302±37 ng/mg), while right atrial (RA) ANF content was significantly reduced (440±45 vs 601±58 ng/mg). At this time, plasma ANF concentration was significantly elevated compared to controls (238±107 pg/ml vs 101±10 pg/ml). These results suggest that the development of pulmonary hypertension following chronic hypobaric exposure induces altered atrial ANF content and increased plasma ANF concentration as a result of altered distension of the atrial wall.

Introduction. Mammalian atrial myocytes possess numerous secretory-like granules which have recently been demonstrated immunocytochemically to contain a family of peptides referred to collectively as atrial natriuretic factors (ANF) (1). ANF has potent natriuretic, diuretic and vasorelaxant properties (2-5). The recent demonstration that ANF concentration in rat plasma is significantly increased by sodium overload (6) strongly suggests that ANF released from the atria plays a hormonal role in the regulation of blood volume.

Several factors may regulate the release of ANF from atria including stimulation of cholinergic, adrenergic or vasopressin receptors on atrial myocytes (7). Physical distension of the atrial walls may also provide a direct, or indirect,

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stimulus for the release of ANF. Strong support for this hypothesis was recently provided by Dietz (8) who demonstrated increased release of ANF from the hearts of rats with atria distended by increased venous return pressure.

In the present study, we have investigated the effects of the development of pulmonary hypertension on the levels of atrial and plasma ANF. This system was chosen because its effects are limited primarily to the pulmonary circulation and therefore any observed changes in atrial or plasma ANF values could be directly related to known or hypothesized changes in atrial pressure. Atrial and plasma ANF values were determined by specific radioimmunoassay (6) using an antibody against rat ANF-IV (9). The development of pulmonary hypertension resulted ultimately in increased release of ANF from the right atrium as demonstrated by decreased right atrial ANF content and increased plasma ANF concentration after 21 days.

Materials and Methods. Male Sprague-Dawley rats, 175-200 g, were exposed to chronic hypobaric hypoxia (0.5 atm) for 3 or 21 days as previously described (10). Pair-fed rats, housed in open hypobaric chambers, served as controls. On the day of sacrifice rats were removed from the chambers, anesthetized with nembutal and blood was withdrawn from the abdominal aorta for determination of hematocrit and plasma ANF. Blood for ANF determination (3 ml) was mixed with aprotinin (final concentration 500 KIU/ml) and EDTA (1 mg/ml) and plasma was stored at -70°C. After midline thoracotomy, rats were perfused via the left ventricle with 200 ml Tyrode's buffer, pH 7.2. Left and right atria were removed from approximately 1/2 of the rats at this time and frozen at -70°C. The ventricles were removed and kept in 2% glutaraldehyde (4°C) for determination of right ventricle (RV) to left ventricle plus septum (LV+S) weight ratios (RV/LV+S). The remainder of the rats were perfused with cold Perfix (Fisher) and left and right atria were removed and processed for light microscopic immunocytochemistry.

Radioimmunoassay for ANF in atria and plasma samples were performed as previously described (6). Briefly, antibody was raised against synthetic rat ANF-IV (11) coupled to bovine thyroglobulin and injected intradermally in Dutch Belted rabbits (6). Atria were homogenized in 2 ml of 0.1N HCl and centrifuged at 10,000 x g for 60 min at 4°C. Following lyophilization, the supernatants were reconstituted in 1 M acetic acid and heated in boiling H_2O for 10 min. The samples were again lyophilized and reconstituted in standard diluent (0.1 M Tris-acetate buffer, pH 7.2, containing 0.1% bovine serum albumin, aprotinin 500 KIU/ml), soybean trypsin inhibitor (50 BAEE units/ml) and 0.02% sodium azide. Plasma samples (1 ml) were applied to a Sep-Pak C_{18} cartridge and absorbed ANF was eluted with 3 ml of 80% methanol. The eluents were evaporated under nitrogen, lyophilized and reconstituted in standard diluent. One hundred µl of samples or standard ANF IV were incubated with 500µl of standard diluent, 100µl of antiserum (final dilution 1:50,000) and 100µl of ^{125}I-ANF (120 µCi/µg) at 4°C for 48 hrs. Separation of antibody- bound and free ANF was accomplished by the addition of 800 µl of a dextran- coated charcoal solution. Duplicate samples were counted in a Beckman Gamma 4000 counter. Groups were compared by a t-test and multiple comparisons performed by the Bonferroni modification.

Light microscopic immunocytochemistry was performed using the avidin-biotinylated peroxidase complex (ABC) technique (Vector Laboratories, Burlingame, CA) as described previously (12). Sections incubated with normal rabbit serum or antiserum absorbed with excess ANF-IV (10 µg/ml) served as controls.

Results. There were no significant differences in body weight between hypoxic rats and their respective controls after either 3 or 21 days (Table I). Hematocrit values for control and hypoxic rats are shown in Table I. Values for control rats at 3 and 21 days were not significantly different. Hematocrit was significantly elevated in both 3 day and 21 day hypoxic rats compared to controls. The ratios of RV/LV+S for 3 and 21 day control rats were not significantly different. Three days of hypoxia produced significant RVH which increased dramatically by 21 days (Table I).

Atrial ANF values are depicted in Table I. ANF contents of the left and right atria from 3 day hypoxic rats were not significantly different from those of

TABLE I. Development of pulmonary hypertension and associated effects on atrial and plasma ANF concentrations †

DAY	BW	HEM	RV/LV+S	RA-ANF	LA-ANF	P-ANF	RA/LA
3C	186±4 g	46±1%	0.249±0.006	548±144 ng/mg	449±74 ng/mg	101±10 pg/ml[a]	1.22
	(8)	(8)	(7)	(3)	(3)	(7)	
3H	181±3	50±1%**	0.278±0.005****	491±88	353±46	149±113	1.39
	(8)	(7)	(8)	(6)	(5)	(3)	
21C	264±6	46±1%	0.222±0.007	601±58	302±37	101±10[a]	1.99
	(4)	(8)	(7)	(3)	(3)	(7)	
21H	246±8	70±3%***	0.536±0.021****	440±45*	508±70**	238±107*	0.87
	(13)	(6)	(8)	(6)	(5)	(8)	

BW = body weight, HEM = hematocrit, RV/LV+S = ratio of weights of right ventricle to left ventricle + septum, RA-ANF = right atrial ANF content (mg ANF/mg tissue), LA-ANF = left atrial ANF content, P-ANF = plasma ANF concentration (pg ANF/ml plasma), RA/LA = ratio of atrial ANF contents, number of rats in ( ).

*$p<0.05$, **$p<0.025$, ***$p<0.005$, ****$p<0.0005$ vs controls

a = combined 3 and 21 day controls

† = All values represent mean ± SEM

controls. However, after 21 days, ANF content of left atria from hypoxic rats was significantly elevated compared to controls ($p < 0.025$) while ANF content of the right atria in hypoxic rats was significantly reduced ($p < 0.05$). Hypoxic exposure resulted in a significant reversal of the right/left ANF ratio which is greater than 1 in normal rats (Table I). Plasma ANF content was slightly elevated in 3 day hypoxic rats and was significantly increased in 21 day hypoxic rats ($p < 0.05$) (Table I). Immunocytochemistry demonstrated intense paranuclear staining for ANF in atrial myocytes of samples from all experimental and control groups but failed to clearly demonstrate differences in ANF content as determined by radioimmunoassay (not shown).

Discussion. Exposure of rats to chronic hypobaric hypoxia is a well known method for modelling the onset, development and establishment of pulmonary hypertension (10, 13, 14). In the present study we have demonstrated increased plasma levels of ANF in conjunction with established pulmonary hypertension at 21 days as indicated by significant RVH which parallels increased right ventricular pressure in hypoxic rats (10). The plasma ANF levels found in control rats in the present study were very similar to those reported previously (6). In addition, we have demonstrated significant changes in the atrial contents of ANF in the established phase of pulmonary hypertension, including a reversal of the normal right-to-left atrial ratio of ANF content (6, 15).

Changes in the atrial and plasma ANF concentrations have previously been noted in experiments where blood volume was altered by high or low salt diets (6, 16). When 1% NaCl was substituted for drinking water for 2 weeks, experimental rats demonstrated approximately a 1.8 fold increase in the ANF content of both

the left and right atria and approximately a 2.3 fold increase in plasma ANF. These results are indicative of an increased synthesis and release of ANF in response to sodium-induced increase in blood volume (6). The latter results also suggest that synthesis and release of ANF may be regulated by changes in pressure-induced distension of the atrial walls. Cantin et al. (1) have discussed the role of pressure-induced atrial distension in maintaining the apparently greater amount of ANF in the right vs left atrium as deduced by differences in immunocytochemical staining intensity. They have also correlated increased granularity in atrial myocytes (presumably resulting from decreased secretion) with decreased blood volume produced by sodium depletion or dehydration. In addition, elevation of central venous return pressure to isolated rat heart-lung preparations has been shown to significantly increase the ANF activity of perfusate samples injected into normal rats (8). The results of the present study further confirm the relationship between increased atrial distension and increased ANF release.

In the present study, the 2.4 fold increase in plasma ANF in rats with well established pulmonary hypertension (21 days hypobaric exposure) strongly suggests stimulation of atrial ANF release by increased right atrial distension. Increased right atrial pressures are commonly found in experimental animals with severe pulmonary hypertension leading to right heart failure (17 - 19). The development of right heart failure induced by primary pulmonary hypertension in man is also associated with increased jugular venous return pressure and right atrial enlargement (20). The significant decrease in right atrial ANF content of rats exposed to chronic hypoxia for 21 days may therefore be explained by the chronic depletion of atrial ANF by continued atrial distension. The increased ANF content in the left atrium of rats with established pulmonary hypertension (21 days) suggests accumulation of synthesized ANF and inhibited release of ANF due to lowered pulmonary venous return pressure.

It is presently unknown whether atrial distension directly stimulates ANF release or whether intermediate steps may be involved. Sonnenberg and Veress (7) have suggested a possible pathway of neurally mediated stimulation of ANF secretion involving the Bainbridge reflex. In this system, distension of the atrial walls would activate atrial stretch receptors with vagal afferents. The increased activity would result in the activation of cardiac sympathetic nerves leading to ANF release mediated by alpha-receptors (7). It has been demonstrated that adrenaline, acetylcholine and arginine-vasopressin stimulate the release of ANF from atrial tissue incubated in vitro and that the common link between these agents is stimulation of the intracellular polyphosphoinositide system (7). In apparent contradiction to this indirect pathway, a recent study by Ledsome et al. (21) demonstrated increased plasma ANF of cardiac origin following mitral obstruction in anesthetized dogs. Their data corroborate a direct relationship between release of ANF and stretch of the atrial wall. In conclusion, the results of the present study demonstrate a positive relationship between the development of pulmonary hypertension and increased atrial secretion of ANF. It remains to be shown whether the natriuretic-diuretic and vasorelaxant activities of ANF may play any role in the moderation of the pulmonary hypertensive response to chronic hypobaric exposure.

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