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CORRELATION BETWEEN CARDIAC HYPERTROPHY AND PLASMA LEVELS OF ATRIAL NATRIURETIC FACTOR IN NON-SPONTANEOUS MODELS OF HYPERTENSION IN THE RAT

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ABSTRACT: We have compared atrial and plasma concentration of atrial natriuretic factor (ANF) in 4 models of non spontaneous experimental hypertension with different pathogenic mechanisms in the rat: two-kidney, one-clip (2-K, 1-C), one-kidney, one-clip (1-K, 1-C), DOCA-NaCl and adrenal regeneration hypertension (ARH) and their respective normotensive controls. All hypertensive groups developed cardiac hypertrophy. In all hypertensive groups plasma ANF was higher than in controls. Atrial ANF concentration was lower in the right and left atrium of 1-K, 1-C rats and in the left atrium of ARH. A good correlation was found between systolic BP and plasma ANF in 2-K, 1-C ($r=0.82$; $p < 0.01$) and 1-K, 1-C animals ($r=0.70$; $p < 0.01$). This correlation was less good in DOCA-NaCl ($r=0.41$; $p < 0.05$) and non existent in ARH ($r=0.28$; NS). A negative correlation between plasma ANF and atrial ANF concentrations was found only in the 1-K, 1-C group ($r=0.41$; $p < 0.05$). A good correlation between plasma ANF levels and cardiac weight was found in all groups: 2-K, 1-C ($r=0.83$; $p < 0.01$), 1-K, 1-C ($r=0.73$; $p < 0.01$), DOCA-NaCl ($r=0.69$; $p < 0.01$) and ARH ($r=0.71$; $p < 0.01$). We suggest that the release of ANF in experimental hypertension depends of the pathogenesis and could be related either to the level of BP (hence the magnitude of the left ventricular end-diastolic pressure) or to the existance of an expanded blood volume. The correlation between plasma ANF levels and cardiac hypertrophy suggests that ANF could be partially released by the ventricles.

Experimental hypertensive rats have been described as having varying concentration of atrial natriuretic factor (ANF) in atrial, plasma, or both. A decrease in bioassayable atrial ANF was reported in spontaneously hypertensive rats (SHR, 1), finding which was later confirmed by a specific radioimmunoassay for ANF (2). Higher levels of plasma ANF have been reported in the same strain in coincidence with the onset of high blood pressure (3-5). Another model of spontaneous hypertension which has received ample attention

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is the Dahl salt-sensitive hypertensive rat. Either a high (6, 7) or unchanged (8, 9) content of atrial ANF have been noted. Plasma levels of ANF are elevated (8-10).

Nonspontaneous models of experimental hypertension have received less attention. DOCA-salt hypertension has been recently reported as having high plasma ANF levels (11, 12) and a lower content of atrial ANF (both atria taken together). One-kidney, one-clip (1-K, 1-C) hypertensive rats have high plasma ANF concentration early during the development of high blood pressure and low atrial ANF content depending of the stage of development (13). Unchanged levels of atrial ANF have been reported in two-kidney, one-clip rats (2-K, 1-C) (14).

It seems that all models of experimental hypertension heretofore studied present changes in either atrial or plasma ANF. In order to verify whether this is true independently of the underlying causes of high blood pressure, we have now investigated nonspontaneous models of experimental hypertension with different pathogenetic mechanisms.

MATERIALS AND METHODS

Hypertensive Animals

2-K, 1-C hypertension was produced in male Sprague-Dawley rats (180-200 g) by constriction of the left renal artery with a silver clip having an internal gap of 0.20 min under anesthesia (sodium pentobarbital, 60 mg/Kg i.p.), the contralateral kidney was left untouched. 1-K, 1-C hypertensive rats were similarly prepared, except that a week later the contralateral kidney was removed. Two groups were used as normotensive controls; the first was subjected to a sham operation in which the left kidney was exposed and the renal artery striped of surrounding tissue (2-K, 1-C controls), the second was subjected to a right nephrectomy (1-K, 1-C controls). The animals were kept on regular rat chow and tap water "ad libitum".

DOCA-salt hypertension was induced in male Sprague-Dawley rats (180-200 g) by implanting subcutaneously a pellet containing 25 mg of deoxycorticosterone acetate (DOCA, Innovative Research of America, Scarborough, Ontario). Controls were sham-operated. Both groups, DOCA-salt and controls, were uninephrectomized and kept on regular rat chow and 1% NaCl.

Adrenal regeneration hypertension (ARH): Since male rats are refractory to develope this model of hypertension (15), female Sprague-Dawley rats (180-200 g) were used. Through a plank incision both adrenal glands were exposed, the right adrenal and right kidney were removed; the left adrenal capsule was slit and the gland gently squeezed out with a forceps. The control group was subjected to right nephrectomy and adrenalectomy and the left adrenal gland was gently manipulated. Both groups were kept with regular rat chow and 1% NaCl as drinking fluid.

Systolic blood pressure (BP) was measured indirectely twice a week by means of a tail cuff under light ether anesthesia, and recorded on a grass model 7 polygraph fitted with a 7P8 preamplifier and a model 1010 Grass crystal microphone as a pulse detector. 2-K, 1-C, 1-K, 1-C and DOCA-NaCl rats were considered hypertensive when their systolic BP was consistently 150 mmHg or higher. Because adrenal enucleated rats tend to develop lower levels of blood pressure than the other models, their BP to be considered hypertension, has been set to 140 mmHg (notice, however, that they also developed cardiac hypertrophy).

Since the lag between surgical operation and the rising of BP is dissimilar in the different models of hypertension, we have normalized the different groups by starting the experiments after 4 weeks of consistently having BP of 140 mmHg (ARH), or 150 mmHg (2-K, 1-C, 1-K, 1-C, DOCA-NaCl) or higher.

Once the animals were hypertensive for 4 weeks blood was withdrawn under pentobarbital anesthesia (60 mg/kg i.p.) by jugular vein puncture. Blood was collected in glass tubes containing the following protease inhibitors at final concentrations of; 1.0×10^{-3} M EDTA; 5×10^{-6} M pepstatin; 1000 U/ml aprotinin; and 3×10^{-5} M phenylmethylsulfonyl fluoride (PMSF). The samples were immediately centrifuged at 2000 g for 10 min at 6°C. ANF was extracted from plasma with Vycor glass beads (Corning Glass Works, Corning, NY) and measured by radioimmunoassay (RIA) (16).

Hearts were rapidly removed and right and left atria dissected separately. Atrial ANF content was measured by RIA (17). Atrial protein content was assessed by a modification of Bradford's method (18).

The data are expressed as means ± SEM and were analysed by the unpaired Student's t-test and considered significant at $p < 0.05$.

RESULTS

Figure 1 depicts BP (A, upper panel), atrial ANF concentrations (B, middle panel) and plasma ANF levels (C, lower panel) in the four models of hypertension presently studied. BP was higher in 2-K, 1-C (189 ± 5 mmHg) and 1-K, 1-C (173 ± 4 mmHg) than in DOCA-NaCl (153 ± 2 mmHg) or ARH (143 ± 2 mmHg). Atrial ANF concentrations were lower in both the right and left atrium of 1-K, 1-C rats, and in the left atrium of ARH animals than in their respective normotensive controls. No differences were observed in atrial ANF in 2K-1C or DOCA-NaCl animals.

Plasma ANF levels were higher in all hypertensive models than in their normotensive controls, but plasma ANF in both controls and ARH rats were lower than in the other groups.

Table 1 illustrates body and heart weights, and hematocrit in all groups. Body weight was lower in renovascular hypertensive groups (2-K, 1-C and 1-K, 1-C) than in their controls. No difference in body weight was observed in DOCA-NaCl or ARH animals. All hypertensive animals presented a relative heart weight higher than their normotensive controls. Hematocrit

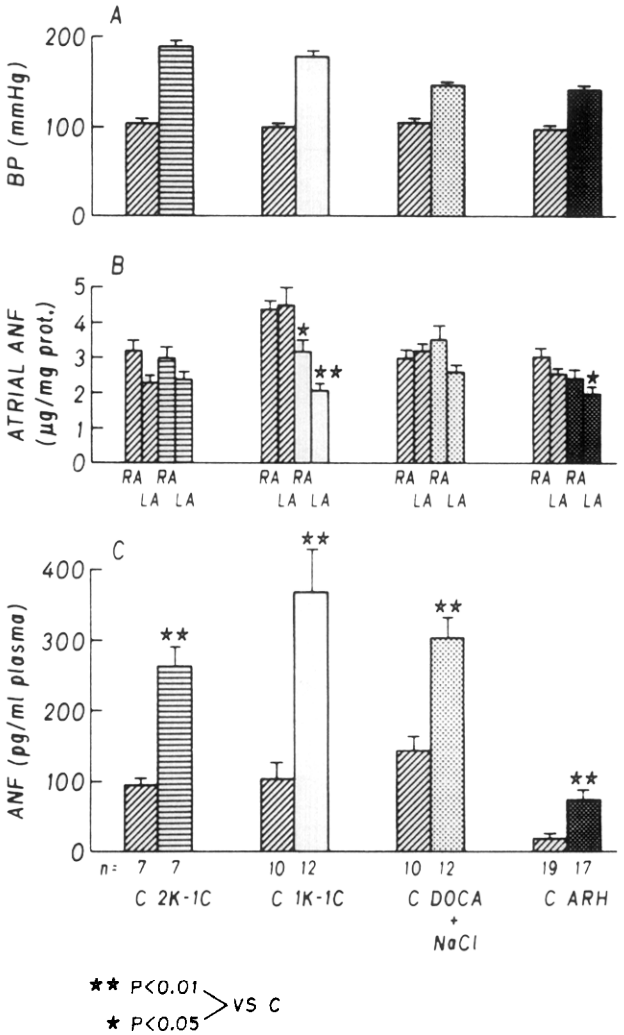

Figure 1 BP (upper panel), atrial ANF concentration (middle panel) and plasma ANF levels (lower panel) in 4 models of experimental hypertension in the rat. RA= Right atrium; LA = left atrium; 0 = number of animals.

was higher in 2-K, 1-C animals and lower in ARH than in their respective normotensive controls.

When both groups, hypertensive and normotensive, for each model of hypertension were taken together a very significant correlation was observed between systolic BP and plasma ANF concentration in 2-K, 1-C ($r=0.82$; $p < 0.01$) and 1K-1C ($r=0.70$; $p < 0.01$). A significant but poor correlation between those two parameters was observed in DOCA-salt ($r=0.41$; $p < 0.05$) and none in ARH animals. A rather poor but significant negative correlation was observed between plasma ANF and atrial ANF concentration in 1-K, 1-C animals

TABLE 1

BODY AND HEART WEIGHTS, AND HEMATOCRIT IN

4 MODELS OF EXPERIMENTAL HYPERTENSION IN THE RAT

Group	Body weight (g)	Heart weight (mg/100 g body weight)	Hematocrit (%)
2-K, 1-C n=7	326 ± 26 *	438 ± 27 **	44.1 ± 0.5 **
Control n=7	402 ± 12	269 ± 4	40.6 ± 0.4
1-K, 1-C n=12	306 ± 11 *	451 ± 18 **	41.5 ± 0.5
Control n=10	332 ± 5	312 ± 7	39.9 ± 0.7
DOCA-NaCl n=18	367 ± 9	366 ± 9 **	38.9 ± 0.4
Control n=10	365 ± 10	281 ± 7	40.8 ± 0.8
ARH n=17	332 ± 9	357 ± 18 *	38.1 ± 0.8 *
Control n=19	345 ± 7	313 ± 10	40.1 ± 0.4

Values are mean ± SEM

* $p < 0.05$

** $p < 0.01$ vs control

(r=-0.41; $p < 0.05$). A good correlation was observed, in all groups investigated, between plasma ANF and relative heart weight (Table 2).

DISCUSSION

We have previously established that a nonspontaneous model of experimental hypertension such as the 1-K, 1-C presented elevated levels of plasma ANF early during the development of high blood pressure (13). 1-K, 1-C has been classically considered as a volume-dependent hypertension (19, 20). On the other hand, another renovascular hypertension, 2-K, 1-C, may or not be a renin-dependent model, since during the development of high blood pressure not all clipped rats necessarily develop renin dependency (21, 22). When

TABLE 2

CORRELATIONS BETWEEN PLASMA ANF CONCENTRATION AND BP, LEFT ATRIAL ANF CONCENTRATION AND HEART WEIGHT

Group	BP (mmHg) vs Plasma ANF (pg/ml)	Plasma ANF (pg/ml) vs LA ANF (µg/mg.prot)	Plasma ANF (pg/ml) vs Heart weight (mg/100 g BW)
2-K, 1-C	0.82 **	0.13	0.83 **
1-K, 1-C	0.70 **	- 0.41 *	0.73 **
DOCA-NaCl	0.41 *	0.12	0.69 **
ARH	0.28	- 0.29	0.71 **
All groups	0.58 **	0.15	0.57 **

* $p < 0.05$

** $p < 0.01$

LA = left atria

systolic BP is over 180 mmHg, 2-K, 1-C rats may have a negative sodium balance and a reduced blood volume (23). DOCA-NaCl is another model, like 1-K, 1-C rats, with a extracellular fluid volume expansion, this time mineralocorticoid-induced (24). The cause of the rise of BP in ARH is not well known. It has been reported that adrenal enucleated rats present a positive sodium balance between 3 and 8 days after surgery (25, 26). However, 3 weeks later their ability to excrete sodium has been established (25, 26), and the animals which became hypertensive no longer have a positive sodium balance (27). A mineralocorticoid, known not to be aldosterone (28) has been suggestive as the cause of the early sodium retention. Another putative candidate, DOC, has been reported to be increased during the 2nd and 3rd week following adrenal enucleation, to drop, afterwards, to normal levels. However, BP persisted elevated after that period (29). Whether ARH corresponds to be a metacorticoid hypertension (30) remains to be determined.

We have now demonstrated that independently of these mechanisms underlying the rise in BP, all hypertensive rats presented higher plasma ANF levels than their respective normotensive controls. All hypertensive animals presented cardiac hypertrophy, substantiating their hypertension, even the groups, such as DOCA-NaCl and ARH, with lower levels of BP.

2-K, 1-C animals with a mean BP of 189 ± 5 mmHg presented a significantly lower body weight and significantly higher hematocrit than their normotensive counterparts, suggesting, like it has been demonstrated by other (23), a negative sodium balance and retracted plasma volume. The stimulus for ANF release in this model of hypertension with a contracted extracellular volume could be the high blood pressure by itself, which by rising left ventricular end-diastolic pressure will rise left atrial pressure, hence stretching the atrium a known mechanism for ANF release. This hypothesis has been supported by the recent findings in our laboratory that infusion of angiotensin II into conscious rats is followed by ANF release only when it is accompanied by a rise in BP and in left ventricular end-diastolic pressure, even in the absence of any change in right atrial pressure (Lachance, Garcia, unpublished results). Non pressive doses of Angio II did not modify ANF plasma levels, suggesting that 2-K, 1-C rats may have increased plasma ANF not because the later is stimulated by high levels of plasma angiotensin II, but because of the hypertension by itself. Similarly to previous results (14), we did not find differences in atrial ANF content between normotensive and 2-K, 1-C rats.

The high levels of plasma ANF observed in 1-K, 1-C rats could be secondary to increased intra-atrial pressure by a dual mechanism. One, like in the 2-K, 1-C animals is the rise in BP, which by increasing end-diastolic filling pressure will rise left atrial pressure, and the other could be the expanded blood volume known to exist in 1-K, 1-C rats (19, 20) and a known stimulus of ANF release (31). The good correlation observed between plasma ANF and systolic BP in 2-K, 1-C rats and their controls ($r=0.82$; $p < 0.01$) suggests that in this model, high BP could be the main stimulus of ANF release. In 1-K, 1-C rats, the same correlation, however significant, is lower than in the previous model, suggesting that even if high BP could be an important stimulus of ANF release, another factor (volume expansion) could be also involved.

On the other hand, DOCA-NaCl animals presented a poor, albeit significant correlation between plasma ANF and BP levels ($r=0.42$; $p < 0.05$), suggesting that in this case other factors (i.e. volume expansion) may play a more important role that BP in stimulating ANF release.

In ARH and controls the levels of plasma ANF are lower than in the other 3 groups. We do not have a clear explanation for this difference. It does not correspond to an atrial ANF depletion, since ANF tissular concentration is not different from the other groups. It could be sex-related, since female rats were used to develope ARH. Another possibility is that during the adrenal gland manipulation, the gland circulation has been damaged producing a hormonal deficiency, and since glucocorticoids are involved in the synthesis and release of ANF (32), the latter could be impaired. Whatever the explanation may be, plasma ANF in ARH is significantly higher than in controls and those levels in contrast with the other models, are not correlated with the manitude of BP. This finding suggests that in this model of experimental hypertension the level of BP may not be the major factor in ANF release. Since ARH present a lower hematocrit than controls, a volume expansion or unkonwn factors could be the main stimulus.

Atrial ANF concentration was lower in the right and left atrium in 1-K, 1-C rats and in the left atrium of ARH animals. We did not see any difference in DOCA-NaCl rats, in contrast with other investigators (11). The atrial levels of ANF in experimental hypertension may depend on whether there is a preponderance of volume expansion on high BP or vice versa, which may be reflected in ANF concentrations in either the right or left atrium. They may also be quite inconstant depending of the time during the development of hypertension in which the observations were made (13). A negative correlation between the concentrations of plasma and atrial ANF was observed only in 1-K, 1-C rats, suggesting a tissular ANF depletion. An unexpected observation was the significant correlation observed between plasma ANF and the degree of cardiac hypertrophy, even in models such as DOCA-NaCl or ARH where a poor or no correlation between plasma ANF and the magnitude of systolic BP

was observed. It has been recently reported that the content of ANF in the hypertropic heart of spontaneously hypertensive rats is much higher than in their normotensive controls (33, 34). This finding together with the close correlation between plasma ANF and cardiac hypertrophy, suggest that ANF under certain circumstances could be released not only by the atrium, but by the ventricle as well. Whether the increased release of this hypotensive peptide is intended as a compensatory mechanism requires further investigation.

In summary, we have demonstrated that the release of ANF is enhanced in several models of experimental hypertension regardless the mechanism involved in rising blood pressure. Plasma ANF may or not be correlated with the magnitude of BP, finding which could be related with a preponderance of BP over volume expansion. Plasma ANF levels, whatever the model of experimental hypertension, were well correlated with cardiac weight, suggesting that the ventricles, once hypertrophic, may participate in ANF release.

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