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# ATRIAL NATRIURETIC PEPTIDE HORMONAL SYSTEM IN PLANTS

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SUMMARY: To determine if atrial natriuretic peptides are present in plants as well as animals, where they are important for water and sodium metabolism, the leaves and stems of the Florida Beauty (Dracena godseffiana) were examined. The N-terminus consisting of amino acids (a.a.) 1-98 (i.e., pro ANF 1-98), the mid portion of the N-terminus (a.a. 31-67; pro ANF 31-67), and C-terminus (a.a. 99-126; ANF) of the 126 a.a. atrial natriuretic factor (ANF) prohormone were all present in the leaves and stems of this plant. The concentrations of pro ANF 1-98, pro ANF 31-67 and ANF-like peptides of 120±20, 123± 21, and 129±20 ng/g of plant tissue in leaves and 109±20, 96±21, and 124±18 ng/g of tissue, respectively, in the stems were lower ($P<0.05$) than their concentrations in rat (Rattus norvegicus) heart atria of 196±40, 192±28, and 189±15 ng/g of tissue respectively, but higher ($P<0.001$) than their respective concentrations of 4.3±1.4, 4.1±1.2, and 3.9±1 ng/g of rat heart ventricular tissue. We conclude that the atrial natriuretic peptide-like hormonal system is present in the plant kingdom as well as in the animal kingdom.



The movement of water and nutrients from the roots up through stems to the leaves in all plants occurs in the xylem, which is composed of two functional systems for conducting water and nutrients upward (1). One conduit system consists of a series of vessels connected end-to-end by perforation plates (1). A second functional conduit for water in xylem is composed of non-living, non-perforated cells connected end-to-end (ie, trachied cells) (1). What controls the movement of water in plants is unknown.

In humans and animals the newly described atrial natriuretic peptide hormonal system appears to play an important role in water and sodium movement and excretion (2,3). This hormonal system consists of a 126 amino acid (a.a) prohormone which circulates as a 98 a.a. N-terminus (pro ANF 1-98) and a C-terminus consisting of a.a. 99-126 in humans (4-17) and animals (3,18-20). Atrial

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natriuretic factor (ANF), the 28 a.a. carboxy (C)-terminal end of this prohormone, and at least 2 peptides from the N-terminus of the ANF prohormone consisting of a.a. 1-30 (pro ANF 1-30; Long acting sodium stimulator) and a.a. 31-67 (pro ANF 31-67; Vessel dilator) have similar vessel dilating, water, and sodium excreting properties (3,22). Since dilation of vessels in the xylem is thought to move water more rapidly in plants (1), the present investigation was designed to determine if plants have the atrial natriuretic peptide-hormonal system, which might cause these vessels to dilate. The stems and leaves of the Florida Beauty (Dracena godseffiana), a monocotyledon vascular plant, were examined for the presence of the atrial natriuretic peptide-hormonal system utilizing 3 sensitive and specific radioimmunoassays which recognize 1) the whole N-terminus of the ANF prohormone (a.a.1-98), 2) the midportion of the N-terminus (proANF 31-67), and 3) the C-terminus (i.e., a.a. 99-126; ANF).

## MATERIALS AND METHODS

Florida Beauty (Dracena godseffiana), a green and yellow patterned leaf plant, was obtained from Carolina Biological Supply Company, Burlington, North Carolina. Leaves and stems of this plant were examined and their concentrations of atrial natriuretic peptides compared with the respective concentrations of these peptides in heart atria and ventricles of the rat (Rattus norvegicus), 150-200 grams, obtained from Charles River Breeding Laboratory, Wilmington, Massachusetts. Representative stems and leaves as well as heart atria and ventricles were first placed separately in 2 mls of phosphate buffer, pH 7.4, and homogenized with a polytron homogenizer. The homogenized stems and leaves were then sonicated to make their preparations as homogeneous as the animal tissue preparations. Each of the homogenized stems, leaves, atria, and ventricles were centrifuged at 3,000xg for 10 minutes and the supernatant and particulate fractions examined by radioimmunoassay for the presence of atrial natriuretic peptides. Radioimmunoassays to measure the N-terminus of the ANF prohormone were developed to a.a. 1-30 and 31-67 of the 126 a.a. prohormone while the C-terminal assay measures a.a. 99-126 of the prohormone, i.e., ANF, as described previously by our laboratory (11,12). Our proANF 1-30 radioimmunoassay recognizes a 10,000 MW peptide as characterized by G-50 Sephadex gel permeation chromatography which is consistent with the whole N-terminus (i.e., amino acids 1-98), but without the C-terminus attached to it (12). The proANF 31-67 radioimmunoassay recognizes a component of the N-terminus of approximately 3900 MW which is consistent with measuring only proANF 31-67 (3878 MW). Reverse phase high-pressure liquid chromatography using Novapak C-18 (5 micron) cartridge columns revealed that the proANFs 1-30, 31-67, and 99-126 (ANF) measured were authentic.

In the presentation of the data, mean values are followed by the standard error of the mean as an index of dispersion. Data were evaluated statistically by Students' t test for unpaired values. Differences with $p<0.05$ were considered significant.

## RESULTS

The N-terminus of the ANF prohormone (proANF 1-98), proANF 31-67, and ANF were all present in the stems and leaves of the Florida Beauty (Fig 1). The leaves and stems contained similar concentrations of atrial natriuretic factor [ANF, C-terminus of prohormone (Fig. 1)]. Thus, the concentration of ANF in the leaves of the Florida Beauty was 129±20 ng/g of plant tissue versus 124±18 ng/g of tissue in the stems. The concentrations of the N-terminus (proANF 1-98) and proANF 31-67, likewise, were similar in leaves and stems with their concentrations in the leaves of 120±20 ng/g and 123±21 ng/g of tissue respectively being not significantly different from their concentrations in the stems of 96±21 ng/g and 109±23 ng/g of tissue when evaluated by the Students' t test for unpaired values. The ratio of the N-terminus to the C-terminus was 1:1 in both the stems and leaves. Pro ANF 31-67 ratio to the whole N-terminus (proANF 1-98) and to the C-terminus of the ANF prohormone was also 1:1 (Fig. 1).

The concentrations of the N-terminal peptides in the leaves of Dracaena was lower ($P<0.05$) than their concentrations in heart atria, but significantly higher than their concentrations in heart ventricles of mammalian species such as the rat (Rattus norvegicus) (Fig. 2). Thus, the concentration of the 98 a.a. N-terminus of

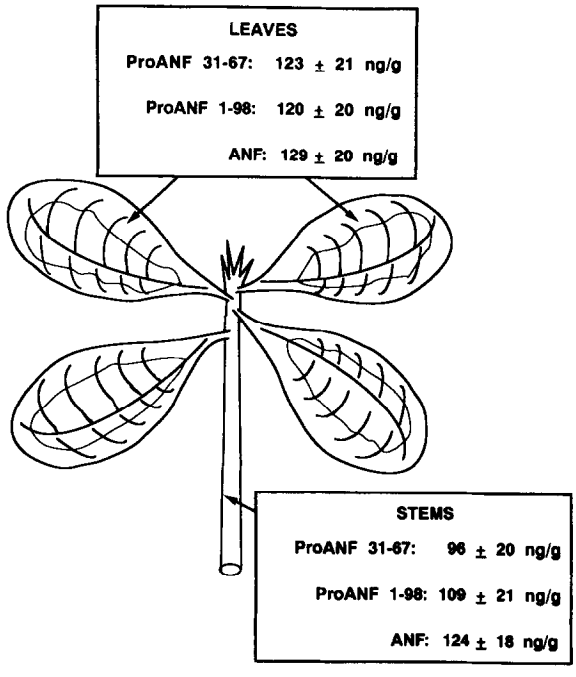


Figure 1. The Florida Beauty (Dracena godseffiana), a monocotyledon vascular plant, contains both the N-terminus (amino acids [a.a.] 1-98), the midportion of the N-terminus (a.a. 31-67) and C-terminus (a.a. 99-126) of the 126 a.a. atrial natriuretic factor prohormone. Both the leaves and stems contained the N-terminus (i.e., pro ANF 1-98), the midportion (i.e., pro ANF 31-67) and C-terminus (i.e. ANF) of this newly described prohormone.

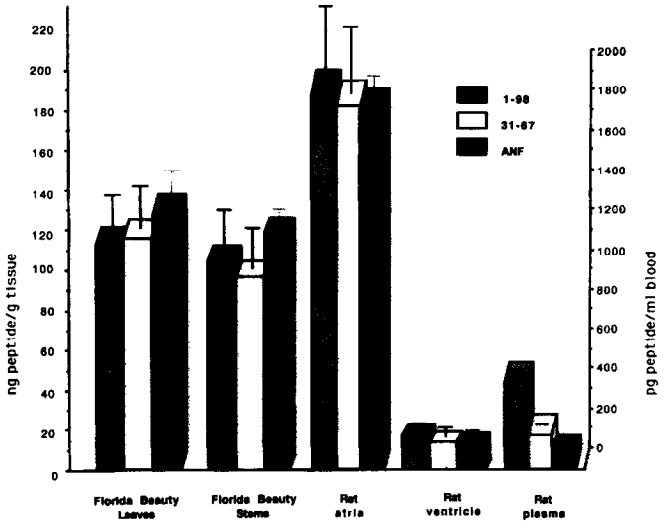


Figure 2. Comparison of the N-terminus (proANF 1-98), midportion of the N-terminus (proANF 31-67) and C-terminus of the atrial natriuretic factor (ANF) prohormone in the stems and leaves of the Florida Beauty (Dracena godseffiana) with their respective concentrations in rat (Rattus norvegicus) heart atria and ventricles (n=10). The respective concentrations of each of these atrial natriuretic peptides in both leaves and stems of Dracena were significantly higher ($P<0.001$) than their concentrations in rat ventricles, but lower ($P<0.05$) than their respective concentrations in rat atria. A comparison of the concentrations of these peptides in leaves and stems of the Florida beauty with their concentrations in rat plasma is also illustrated with the scale for the plasma concentrations being on the right ordinate while the scale for tissue (leaves, stems and rat heart) being on the left ordinate.

120±20 ng/g of leaf tissue was lower than that found in rat heart atria (196±40 ng/g) ($P<0.05$) but significantly higher than that found in rat heart ventricles (4.3±1.4 ng/g) ($P<0.001$). The concentration of proANF 1-98 in the stems of 96±21 ng/g of tissue was also lower ($P<0.05$) then that found in rat atria, but was 22-fold higher ($P<0.001$) than the N-terminal ANF prohormone concentration in rat heart ventricles. Pro ANF 31-67, from the mid-portion of the N-terminus, was also lower ($P<0.05$) in both the leaves (123±22 ng/g of tissue) and stems (124±18 ng/g of tissue) of the Florida Beauty than its concentration in rat heart atria (192±28 ng/g) but significantly higher in both leaves and stems than its concentration in rat heart ventricles (4.1±1.2 ng/g; $P<0.001$). The concentration of proANF 31-67 in both the leaves and stems was 30-fold higher than its concentration in the ventricle of rat heart. (Fig. 2).

The C-terminus of the ANF prohormone was also lower ($P<0.05$) in the stems and leaves of the Florida Beauty versus its concentration in rat atria (189±15 ng/g of tissue). (Fig. 2). The 32-fold higher concentrations of the C-terminus of this prohormone in both stems and leaves than its concentration in rat heart ventricle (3.9±1 ng/g of tissue) were significant at $P<0.001$.

## DISCUSSION

Both the 98 amino acid (a.a.) N-terminus and 28 a.a C-terminus of the atrial natriuretic factor (ANF) prohormone were immunologically recognized to be present in the Florida Beauty suggesting that this plant, as well as animals, is capable of synthesizing an ANF-like prohormone. The amount of the N-terminus, proANF 31-67 and the C-terminus of the ANF-like prohormone in plant leaves and stems was quite remarkable, being at least half as great as the amount found in atria of the heart, the largest single site of ANF prohormone synthesis in animals (2). The slightly larger amount of the ANF prohormone in the leaves versus the stems of the Florida Beauty is possibly due to the slightly larger amount of vessels (veins) present in leaves. The production of the ANF prohormone in plant vessels would not be completely dissimilar from animals, since the largest blood vessel in animals (i.e., the aorta) is second only to atria of the heart in significance as a source of producing the ANF prohormone (2).

The exact function of the atrial natriuretic peptide hormonal system in plants could not be determined from the present investigation but the presence in Dracena godseffiana of the large amounts of the N-terminus and C-terminus of the ANF prohormone with their respective vessel dilatory and water movement properties suggests that these peptides may be important for water movement in plants. Water movement in the stems of plants is known to move faster with a larger diameter of the vessels in the xylem (1). If these peptides do have the ability to dilate vessels in plants, then they could regulate the rate of water movement in plants. The exact role of the atrial natriuretic peptide hormonal system in plant physiology awaits further investigation.

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