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DIFFERENTIAL STRUCTURE-ACTIVITY RELATIONSHIPS OF ATRIAL PEPTIDES AS NATRIURETICS AND RENAL VASODILATORS IN THE DOG

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SUMMARY: Natriuretic-diuretic and vasodilator activities of synthetic atriopeptin (AP)-related peptides were examined in the anesthetized dog. We have selected, the naturally occurring, APIII as the reference compound for comparison with various related peptides. APIII is a 24 amino acid peptide with the sequence ser-ser-cys-phe-gly-gly-arg-ile-asp-arg-ile-gly-ala-gln-ser-gly-leu-gly-cys-asn-ser-phe-arg-tyr-OH. APII, another peptide isolated from atrial extracts, lacks the C-terminal arg- of APIII. N-terminal amino acid extensions on APIII or APII, exhibited enhanced natriuretic-diuretic effectiveness. Furthermore, the maximum response obtained by ser-leu-arg-arg-APIII and arg-arg-APIII were signficantly higher and the dose-response curve was not parallel to that obtained with APIII. In contrast, there were no significant qualitative or quantitative differences between the renal blood flow responses produced by the N-terminal extended peptides and APII or APIII. These results suggest a heterogeneity of AP receptors in vascular and renal tubular tissues.

A series of low molecular weight peptides have been isolated from atrial extracts, and structure elucidation indicated that the reported peptides share the same core sequence, containing a 17 membered ring of amino acids within a cystine disulfide bond, and differ only in the composition of the N- and C-termini (1-8). A high molecular weight fraction (atriopeptigen) was isolated from the atrial extract which was relatively impotent as a spasmolytic agent on isolated smooth muscle preparations. Conversion of the high molecular weight fraction to the smaller highly active peptide was accomplished by gentle proteolysis (9,10). The characterization of the sequence (10-13) and the cDNA (14-17) of the high molecular weight precursor demonstrated that the low molecular weight peptide (atriopeptin-AP) was derived from the C-terminal of the precursor. The multiple low molecular weight atrial peptides therefore are truncated versions of the C-terminus of the precursor and either represent

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the natural secreted form of the peptide or the product of proteolysis that occurred during the isolation from the atrial extracts. Regardless of the source, the low molecular weight peptides are potent agents in altering vascular and renal function. The N-terminal attached to the first cysteine in the core cystine-disulfide ring of amino acids of the atriopeptins isolated from rat atria vary considerably. Atriopeptin I, II and III contain ser-ser at the N-terminal (1,4), others have described arg-ser-ser (RSS) (6,8) arg-arg-ser-ser (RRSS) (7) or ser-leu-arg-arg-ser-ser (SLRRSS) (2) attached to the initial cysteine in the ring. Human atrial peptide also contains SLRRSS- at the N-terminal but the ile within the ring is replaced by a met (3). In the current investigation we performed a structure activity study to compare the quantitative potency of these naturally occurring peptides as natriuretic-diuretic-vasodilators in anesthetized dogs.

METHODS AND MATERIALS

Ten male mongrel dogs weighing 9-14 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and ventilated with room air via a Harvard respirator. The left brachial vein was cannulated for constant infusion of physiological saline containing sodium pentobarbital (1.3 ml/min, 3 mg/kg/hr). Blood pressure was monitored by a Statham pressure transducer via a left femoral arterial cannula. Heart rate was measured by a pulse rate tachometer (Beckman Type 9857B). A polyethylene catherter (PE 160) was inserted into the right ureter through a retroperitoneal flank incision for urine collection. Renal blood flow of the right kidney was measured with an electromagnetic flowmeter (Square wave electromagnetic flowmeter, Carolina Medical Electronics, Inc.). Distal to the flow probe, a 22 guage needle was placed into the renal artery for injection of the test compounds. Following an equilibration period of 60-90 min, five baseline urines were collected at 5 min intervals. The peptide was then injected into the renal artery and urine samples were collected until values returned to baseline, at which time the next dose of peptide was given. Sodium concentrations were determined by flame photometry (IL Model 143). Data were analyzed by linear regression analysis by the method of least square, and results presented are the mean ± SEM. Atriopeptin-(AP)III, APII, met^{8}-APIII, met^{8}-APII, SLRR-APII and RR-APII, SLRR-APIII, RR-APIII and R-APII were prepared by solid phase synthesis.

RESULTS

Fig. 1A and B shows the natriuretic activity of APIII-related and APII-related peptides in anesthetized dogs. Sodium excretion and urine flow rate were increased by APIII and APII in a dose dependent manner. The peak response following the bolus intraarterial peptide injection was observed within 5 min, and lasted less than 15 min. The maximum response ($U_{Na}V$) was

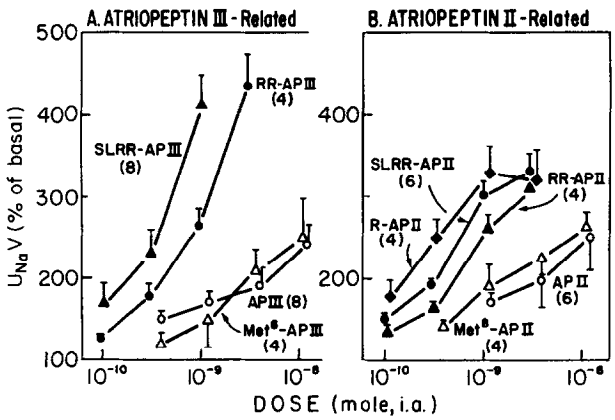

Figure 1. Atriopeptin (AP)-related peptide-induced natriuresis in anesthetized dogs. Following abbreviations were used; SLRR-, and RR- and R- indicate ser-leu-arg-arg-, arg-arg- and arg- N-terminal amino acid extension of atriopeptin, respectively. Each value represents the mean + SE of $U_{Na}V$ relative to control for 5 min collection periods before and after intrarenal arterial administration of peptides. The values in the parenthesis indicate the number of animals used. The basal value for $U_{Na}V$ was 1.76±0.14 μEq/g kidney/min (n=10).

observed at more than 30 nmoles (243±24% for APIII and 263±45% for APII). N-terminal amino acid extended AP-related peptides (SLRR-APIII, SLRR-APII, RR-APIII, RR-APII and R-APII) exhibited more potent natriuretic and diuretic effects than the truncated APIII or APII. The doses required to cause 100% increase in sodium excretion were as follows: 0.14 (SLRR-APIII), 0.38 (SLRR-APII), 0.19 (RR-APIII), 0.60 (RR-APIII), 0.19 (R-APII), 4.9 (APIII) and 2.8 nmoles (APII). The maximum responses obtained by SLRR-APIII (436±38%) and RR-APIII (413±36%) were significantly ($p>0.001$ and <0.005) higher than that of APIII. Switching the eighth amino acid (in rats - ile) to met (position 8 amino acid as in human atrial peptide) did not significantly affect the natriuretic and diuretic responses.

Renal vasodilation induced by AP-related peptides are shown in Fig. 2A and B. The peptides exhibited parallel dose response curves for renal blood flow (measured with a electromagnetic flow probe), but not for natriuresis and diuresis. The dose required to cause an increase of 15 ml/min for APIII (0.69 nmoles) was 2 to 3 times higher than that of SLRR-APIII (0.31) or RR-APIII (0.17 nmoles), but these differences were not significant. At the highest dose tested (up to 3×10^{-8} moles, i.a.), neither blood pressure nor heart rate was affected by intrarenal arterial injection of peptides.

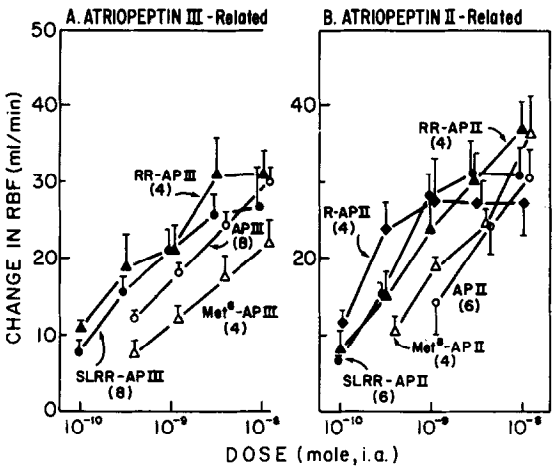

Figure 2. Renal vasodilation elicited by intraarterial administration of atrial peptides in anesthetized dogs. Each value shows the mean ± SE of peak values of renal blood flow (RBF) obtained following intraarterial injection of peptides. The values in the brackets are the number of animals tested. The basal value for RBF was 79±8 ml/min (n=10). Abbreviations used are the same as Fig. 1.

DISCUSSION

In this study we demonstrated that the N-terminal amino acids attached to the first cysteine in the core peptide of atriopeptin (i.e., the 17 membered ring formed by the internal cystine disulfide) quantitatively affected the natriuresis-diuresis and vasodilation produced in the canine kidney following intraarterial injection. N-terminal amino acid extension of APII and APIII (i.e., SLRR-, RR-, and R-) markedly increased natriuretic-diuretic potency while only slightly affecting their renal vasodilator activity compared to truncated peptides. Interestingly, the natriuretic dose response curve for the N-terminal extended peptides are not parallel to the curves obtained with APIII or APII. On the other hand, there was little difference in the slope or the potency of any of the atriopeptins tested as vasodilators. The lack of parallelism between analogs in causing sodium excretion suggests the possibility that the N-terminal extended analogs may affect different renal (possibly tubular) receptors than APII or APIII. Similarly, the differential dose response curves obtained when comparing renal blood flow changes to $U_{Na}V$ suggests the possibility for different receptors (i.e., vascular and tubular) within the dog kidney for two elicited responses. Furthermore, the fact that APIII and APII do not achieve the same maximum response suggests they are

partial agonists. However, it was in fact not possible to test if APIII at much higher doses would match the response of the N-terminal extended APs because of the amounts of material required and because of the pronounced changes in vascular tone that would be produced by larger APIII doses.

The C-terminal amino acids attached to the second cysteine in the core peptide also affected the biological activities. API which has a deletion of the C-terminal phe-arg is essentially inactive in the dog as a renal vasodilator and natriuretic-diuretic. Also removal of the arg (des-arg^{23} APII) markedly decreases the potency as a relaxant of rabbit aorta precontracted by norepinephrine (19). We found that the presence or absence of try^{24} (i.e., APII versus APIII or their related analogs) caused no difference in vasodilator or natriuretic potency. Substitution of the eighth amino acid (ile to met) to evaluate the difference of rat and human atrial peptide did not affect the natriuretic-diuretic potency of atriopeptins.

The current experiment suggests that receptors localized in the intact canine kidney distinguishes differences in N- and C-terminal amino acids of the atriopeptins. The identity of the circulating form of atrial peptide released from the heart has not yet been established. If the plasma peptide contains the N-terminal extended SLRR- (i.e., the 28 amino acid peptide), this would be expected to be more potent, at least in the dog, than APIII. The presence of the arg-arg in the N-terminal sequence of the 28 amino acid peptide predicts a site of enzymatic attack and suggests that APIII may be the smallest likely peptide candidate to possess the full vascular and natriuretic activity following N-terminal proteolysis. This question will be resolved by structural analysis of the plasma form of the peptide.

In this experiment we demonstrated in vivo stuctural dependence of vascular and renal tubular responses which may indicate heterogeneity of receptors in these tissues.

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