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HUMAN PREPRO ATRIAL NATRIURETIC FACTORS 26-55, 56-92, and 104-123 INCREASE RENAL GUANYLATE CYCLASE ACTIVITY

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SUMMARY: Human prepro atrial natriuretic factors 26-55, 56-92, and 104-123 as well as human atrial natriuretic factor (4-28) in the present investigation increased renal cortical and medullary cyclic GMP levels and maximally enhanced particulate guanylate cyclase activity [E.C. 4.6.1.2] two-fold in whole kidney homogenates, renal cortical and medullary membranes, and in isolated distal nephrons at their 1 μM concentrations. Dose-response relationships revealed that the half maximal [ED_{50}] activation of guanylate cyclase was at their 10 nM concentrations in rat, rabbit, and dog kidneys. Both human atrial natriuretic factor and the prepro factors decreased adenylate cyclase activity. These results suggest that prepro factors 26-55, 56-92, 104-123 may also be functionally active.

Myocytes from the atria but not the ventricles of the heart have secretory granules which resemble typical protein secretory cells (1). In 1981, DeBold et al, utilizing crude atrial extracts demonstrated a brisk natriuresis in rats of 30 to 40 fold greater than basal levels (2). It is now known that the peptide(s) which cause this natriuresis have a preprohormone of 151 (human) or 152 (rat) amino acids which lose their hydrophobic leader sequences to form a 126 amino acid prohormone (3-6) (Figure 1). This prohormone is the primary form in which atrial peptides are stored in the perinuclear granules of the atrial myocytes (3-6). The whole 126 amino acid prohormone is released into the circulation (7) and then cleaved by proteases into more than one circulating fragment (8,9). In rat plasma the major circulating form is a low molecular weight carboxy-terminal fragment of 24 amino acids that has been called atrial natriuretic factor (ANF) (8). Atrial natriuretic factor effects

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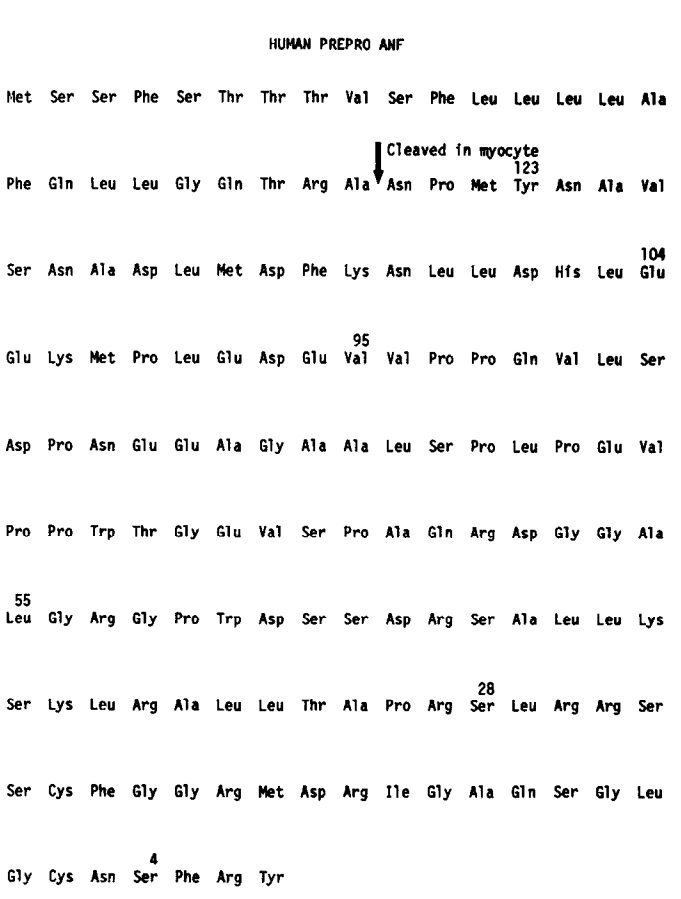

Figure 1. Human prepro atrial natriuretic factor. This 151 amino acid peptide is cleaved in the atrial myocyte (arrow) to produce 126 amino acid peptide which circulates. The numbers above the amino acids refer to their position from the carboxy terminal end which has been designated amino acid number one.

on the kidney have been associated with increased cyclic GMP levels (10-14) and enhancement of particulate guanylate cyclase [E.C. 4.6.1.2] activity (14). To determine whether or not human prepro atrial natriuretic factors have any effects at the cellular level renal cortical and medullary membranes and distal nephrons were isolated. The kidneys utilized in these experiments were harvested from dogs, rabbits, and Sprague-Dawley rats to determine if there was a species specific effect of human ANF or its prepro factors. Human prepro factors 26-55, 56-92, 104-123 and human atrial natriuretic factor (4-28) (Figure 1) were all found to enhance particulate guanylate cyclase activity in the isolated kidney membranes and nephrons from each of the above species.

MATERIALS AND METHODS

Prepro human atrial natriuretic factors 26-55, 56-92 and 104-123 as well as atrial natriuretic polypeptide (human 4-28) were kindly provided by Peninsula Laboratories (Belmont, Calif.) Distal nephrons composed mainly of distal tubule fragments were isolated via the method of Vinay et al (15). After nephrectomy, superficial canine renal cortical slices were incubated with collagenase to obtain a mixture of tubule fragments. This suspension was then separated into proximal and distal fractions by Percoll density gradient centrifugation (15). Renal cortical and medullary membranes were prepared by the method of Lever (16). This method entails careful dissection of the renal cortex and medulla, homogenization, and then a sequence of differential centrifugation and ionic precipitation with 10 mM $MgCl_2$ to separate luminal from basolateral membranes (16).

The distal nephrons, whole kidney homogenates and the renal cortical and medullary membranes were then processed (17) to obtain 105,000 g supernatant and particulate fractions after ultracentrifugation for 60 minutes. The supernatant and particulate fractions were then assayed at 37°C for 10 minutes for guanylate cyclase activity (17). The reaction mixture consisted of 20 mM Tris HCl (pH 7.6), 4 mM $MnCl_2$, 2.67 mM cyclic GMP (used to minimize destruction of (^{32}P)-GMP, a GTP regenerating system (5 mM creatine phosphate and 11.2 U creatine phospnokinase (E.C. 2.7.3.2), 100 µg bovine serum albumin, 20 mM caffeine, and 1.2 mM (α^{32}P)-GTP, approximately $5X10^5$ cpm. The final pH of the reaction mixture was 7.6. The volume of the supernatant or particulate fractions was 25 µl and the final volume of the cyclase assay which included the above supernatant or particulate fractions, the reaction mix and the radioactive isotopes was 75 µl. The enzyme preparations contained 0.1 to 0.2 mg/ml protein. The reaction was terminated by adding 10 µl of 0.1 M EDTA (pH 7.6) containing about 30,000 cpm of (^{3}H)-cyclic GMP (to estimate recovery in the subsequent steps) and boiling for 3 minutes. After cooling in an ice bath, the (^{32}P)-cyclic GMP formed was isolated by sequential chromatography on Dowex 50 Wx4 H^+ (200-400 mesh) and alumina using the modification described in detail previously (17). In this assay system, cyclic GMP production was linear with time for 20 minutes and with added protein from 50 to 400 µg. All of the ^{32}P-containing material was identifiable as cyclic GMP as determined by thin-layer chromatography on PEI-cellulose (Brinkman, Westbury, NY) using 1 M LiCl as solvent and on Chromar sheets (Mallinckrodt Chemical Works, St. Louis, MO) developed with absolute alcohol and concentrated NH_4OH (5:2 v/v). All reagents were of analytical grade and from the same sources as described previously (17). Each experiment was conducted in triplicate, and the results confirmed in six separate experiments. Cyclic GMP and AMP tissue levels were measured by radioimmunoassay (18). Adenylate cyclase activity was measured by similar methods to the guanylate cyclase except that α-32 ATP was utilized instead of α-32-P-GTP (18).

RESULTS

Human prepro atrial natriuretic factors 26-55, 56-92, and 104-123 as well as human atrial polypeptide at their 1 µM concentrations all enhanced particulate guanylate cyclase from 105,000 g whole kidney homogenates, renal cortical and medullary membranes, and in the isolated distal nephrons (Table 1). In isolated renal cortical membranes, atrial natriuretic polypeptide (4-28) also called human ANF was the most potent activator with a four-fold increase in guanylate cyclase activity while the prepro ANF 26-55, 56-92, and

TABLE 1

The effect of prepro ANF (human 26-55, 56-92 and 104-123) as well as ANF (human 4-28) on rabbit kidney particulate guanylate cyclase (105,000 g) activity

	Guanylate cyclase (pmol cyclic GMP per mg protein per 10 minute incubation)				
	No addition	ANF	pp26-55*	pp56-92*	pp104-123*
Whole kidney homogenate	427±29	998±36	842±28	883±36	830±27
Renal cortex membranes	586±32	1951±69	926±37	1055±51	996±34
Renal medulla membranes	532±28	971±39	999±41	961±33	958±30
Distal nephrons**	112±14	167±20	168±18	261±28	184±23

Each value represents the mean ± standard error of the mean (S.E.M.) of triplicate samples determined in six separate experiments. *Prepro ANFs and ANF were tested at their 1 μM concentration. **Distal nephrons were isolated from canine kidneys.

104-123 enhancements were approximately two-fold (Table 1). The prepro ANFs had nearly equal activity to the human ANF in whole kidney homogenates and renal medullary membranes, while in the isolated distal nephrons some of the prepro human ANF's were more active than human ANF itself in enhancing particulate guanylate cyclase activity (Table 1).

Dose response relationships on rabbit renal cortical membranes indicated that prepro ANF's (human 26-55, 56-92, and 104-123) enhanced particulate guanylate cyclase activity to a similar extent throughout their concentration ranges while human ANF was a more potent enhancer of this activity throughout its activity range (Fig. 2). Half-maximal (ED_{50}) stimulation of guanylate cyclase activity occurred at the 10 nM concentration for all the human prepro ANFs as well as for human ANF itself. Both the prepro ANFs and human ANF caused their maximal enhancement of particulate guanylate cyclase at their 1 μM concentrations (Fig. 2). Increasing their concentrations above 1 μM caused no further increase in guanylate cyclase activity. Similar dose-response curves were seen on medullary membranes, whole kidney homogenates, and in isolated nephron segments except that human ANF enhancement of particulate

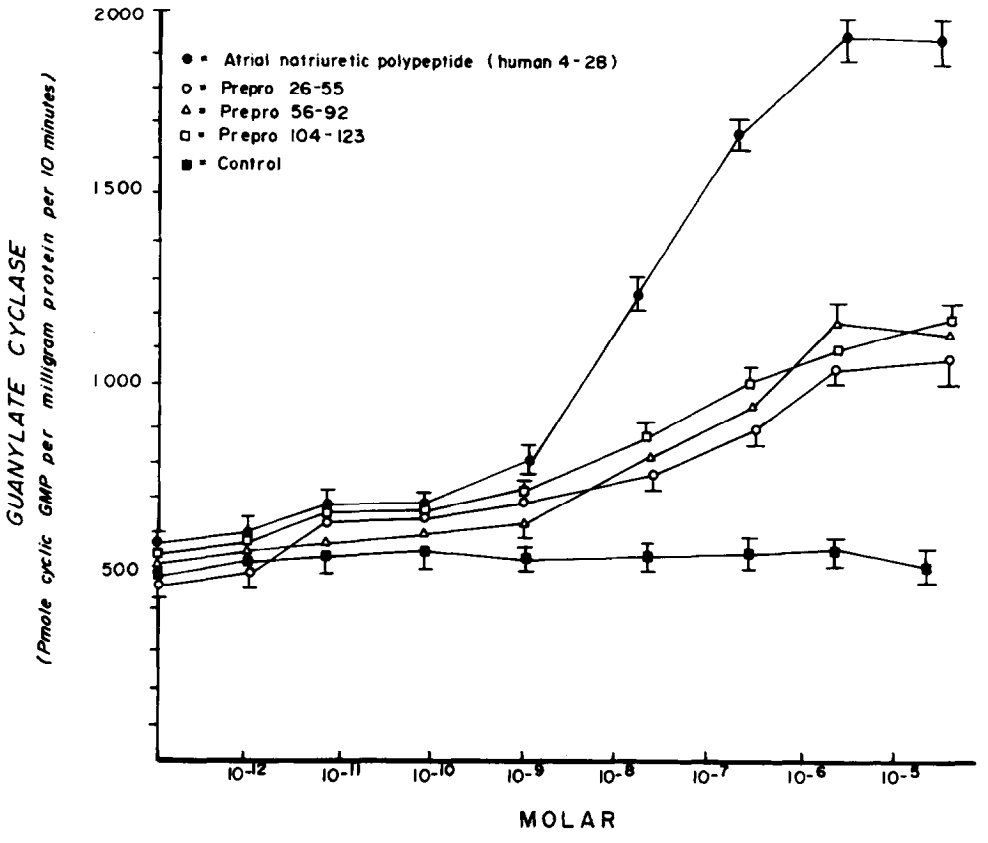

Figure 2. Dose response relationships of human prepro ANF 26-55 (○), 56-92 (△), and 104-123 (□) and human atrial natriuretic polypeptide 4-28 (●) on rabbit renal cortical membranes. Each value is the mean ± S.E.M. of triplicate samples of each group in six separate experiments. The values at 10 nM and above for all of the prepro ANFs and ANF were significant at < .001 compared to control by Student's t test for unpaired values. The control consisted of renal cortical membranes in different molar concentrations of Tris HCl which did not change basal particulate guanylate significantly. The volume of the different molar concentrations was the same (10 µl) for each of the controls and the experimental groups.

guanylate cyclase was not greater than that of the prepro ANFs at any of the concentrations tested.

To determine if there were any species differences with respect to the activation of kidney particulate guanylate cyclase by the various prepro ANFs, their effects were determined in rat, canine, and rabbit kidneys. Human prepro ANFs as well as human ANF had similar effects on renal particulate guanylate cyclase from all three species (Fig. 3). Human ANF and the prepro ANFs did not increase soluble 105,000 g guanylate cyclase in any of these species (Fig. 3). None of the human prepro ANFs or human ANF increased

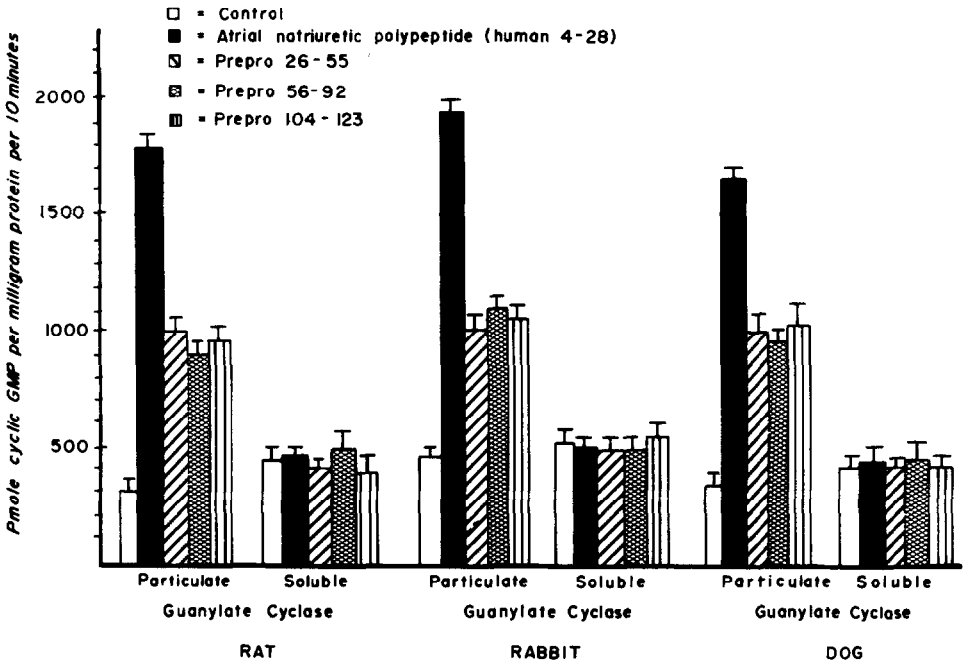

Figure 3. Effect of human atrial polypeptide (4-28), prepro ANF 26-55, prepro ANF 56-92, and prepro ANF 104-123 on particulate and soluble guanylate cyclase activity of renal cortical membranes of rat, rabbit, and canine kidneys. ANF and prepro ANF 26-55, 56-92, and 104-123 enhancement of particulate guanylate cyclase from all three species was significant at $p < .001$ compared to control by the Student's t test for unpaired values.

adenylate cyclase activity. There was, in fact, a slight decrease in adenylate cyclase activity with human ANF and the prepro ANFs (data not shown).

The prepro ANFs as well as human ANF increased cyclic GMP levels in renal cortical and medullary kidney slices. After a 5 minute incubation cyclic GMP levels rose in the renal cortex to 2.79 ± 0.46, 2.83 ± 0.67, 2.76 ± 0.54 and 4.68 ± 0.72 pmol/mg protein for prepro ANF 26-55, 56-92, and 104-123 and human ANF respectively versus a control of 1.37 ± 0.05 pmol/mg protein (mean ± SEM of 12 slices pooled from 6 experiments). For 12 renal medullary slices, the values secondary to prepro ANF 26-55, 56-92, and 104-123 and human ANF were 6.32 ± 0.49, 6.47 ± 0.71, 6.39 ± 0.55, and 6.43 ± 0.66 pmol/mg protein respectively versus a control of 3.03 ± 53 pmol/mg protein. None of the above increased cyclic AMP levels.

DISCUSSION

The present investigation demonstrates that human prepro atrial natriuretic factors 26-55, 56-92, and 104-123 activate particulate guanylate

cyclase and increase cyclic GMP levels in whole kidney homogenates, renal cortical and medullary membranes, and in isolated distal nephrons. This enhancement was nearly equal to that of human atrial polypeptide (4-28) except in renal cortical membranes where ANF was a much stronger activator of guanylate cyclase. This difference in activation may be of significance in that the cortex contains the glomeruli which appear to be the main target of ANF (11-13). Cyclic GMP levels in the glomeruli appear to be increased by ANF more than in proximal or distal tubules (11-13). The present study indicates that in the isolated distal nephron, however, the prepro ANFs are as potent (and slightly more so) than ANF.

The significance of these findings in the isolated distal nephron are unknown at present, but this data does suggest that if any of the prepro atrial peptides are found to have natriuretic effects, these peptides may very well act at different sites than ANF in the kidney. The present data would, furthermore, suggest that if the prepro ANFs do have physiologic effects mediated through the kidney their major effects may be in the distal nephron. The effect of the prepro ANF's on natriuresis is presently being evaluated in our laboratory. The complete 126 amino acid pro ANF which is secreted in the circulation (7) has been shown to have diuretic activity although less than the smaller ANF peptides (19). It would, thus, not be surprising if fragments 26-55, 56-92, and 104-123 are also found to have diuretic and natriuretic properties. Previous studies have demonstrated that more than one peptide fragment circulates for other prohormones such as parathormone and that these fragments have different effects on metabolism (20).

It is of interest that prepro ANF 26-55, 56-92, and 104-123 enhanced particulate but not soluble guanylate cyclase activity. Atrial natriuretic factor is the only known "hormone" to stimulate particulate guanylate cyclase activity only. Most of the other hormones which have been demonstrated to activate guanylate cyclase increase both particulate and soluble guanylate cyclase activity (21-23). Thus, prepro ANF 26-55, 56-92, and 104-123 are also of considerable interest in that they are three new peptides that specifically activate particulate guanylate cyclase.

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