BIOLOGICAL AND MOLECULAR ASPECTS OF ATRIAL FACTORS

Organizer, Philip Needleman
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Biological and Molecular Aspects of Atrial Factors

Keynote Address

A 000 PARADOXICAL RELATIONSHIP BETWEEN ATRIOPEPTIN PLASMA LEVELS AND DIURESIS INDUCED BY VOLUME EXPANSION, M. Sakata, J.E. Greenwald and Philip Needleman, Department of Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.
Surgical appendectomy was employed in rats to reduce the intrinsic stores of atriopeptin. In conscious rats, bilateral or unilateral atrial appendectomy suppressed the diuresis produced by acute volume expansion. On the other hand, the diuresis produced by injection of pharmacologic doses of 1-deamino-arg^8-vasopressin (dAVP) was comparable in the atrialectomized and control groups. Surprisingly, volume expansion did not result in the appearance of plasma atriopeptin immunoreactivity, but administration of dAVP caused comparable elevation of plasma APir (and diuresis) in the control or atrial- appendectomized conscious rats. Previous studies demonstrated that acute volume expansion in anesthetized animals caused increased plasma APir levels. Indeed, we found that volume expansion causes comparable diuresis and natriuresis in conscious and chloral hydrate-anesthetized rats, but that only the latter group exhibits an increase in plasma APir. Brattleboro rats, which are deficient in vasopressin, exhibit the same response as Long-Evans controls in that acute volume expansion in conscious animals produces a pronounced diuresis but no APir release; but when these same animals are anesthetized, there is a simultaneous induction of diuresis and APir release by volume expansion. Neither AP or vasopressin appears to exert a physiologic regulatory role in the diuresis induced by volume expansion in conscious animals. On the other hand, the diuresis induced by acute volume expansion in the anesthetized rat seems dependent on the elevated APir since rats made autoimmune to AP (which are nonresponsive to exogenous AP-24 infusions) only exhibit a diuresis in conscious but not anesthetized rats. We therefore conclude that the participation of AP in volume homeostasis is more likely in pathophysiological states (e.g., excessive pharmacological stimulation, anesthesia) and that another mechanism or possibly another atrial factor mediates the diuresis-natriuresis induced by volume expansion in conscious rats.

Biosynthesis and Regulation

A 001 EXPRESSION OF THE ANF GENE IN TRANSGENIC MICE, Loren J. Field, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 11724. To characterize the expression of the ANF gene in an in vivo system, transgenic mice were generated which carried either the intact human ANF gene, or fusions derived from the human ANF 5' flanking region and a recorder gene. Four independent transgenic lineages with the intact ANF gene were obtained; each lineage expressed transgene mRNA in the atria as demonstrated by Northern blot analysis using a species specific probe. Thus, a functional atrial promoter was present in the microinjected sequences. The expression of the transgene had no effect on the total levels of ANF synthesis, as demonstrated by protein analyses. A second series of transgenic animals were generated which carried fusion genes consisting of ANF 5' regulatory sequences and the SV40 early transcriptional unit (which encodes the large T oncoprotein). The rationale for the use of SV40 T antigen as the recorder gene is two-fold. First, in situ analysis of transgene expression is greatly facilitated due to the unique structure as well as the nuclear localization of the oncopretein. Second, numerous other studies have shown that the expression of T antigen usually results in tumorigenesis in targeted tissues, thereby creating the potential to obtain tumor material with which to generate cell lines. Moreover, T antigen expression might generate an interesting cardiac pathology. Immunohistological studies indicate that transgenic mice which carry the ANF-T antigen fusion gene accumulate oncoprotein in both left and right atrial myocardiocytes in the same spectrum of cell types specified by the endogenous ANF gene. Western blot tissue surveys were performed to ascertain the fidelity of the ANF promoter sequences; expression was only observed in the cardiac atria. However, the sensitivity afforded by these analyses would not detect low levels of transgene expression (or expression in a limited number of cell types). Thus, fusion gene expression in the lungs and brain (which have previously been shown to be secondary siters of ANF synthesis) cannot yet be ruled out. Expression of the oncoprotein in transgenic atria initially has no pathological effect. However, as the animals age there is an interesting pattern of asymmetrical atrial hyperplasia; the right atrium becomes greatly enlarged while the left atrium remains relatively uneffected. Animals with terminal hyperplasia exhibit right atria which are 3-4 fold larger than the entire heart of an age-matched non-transgenic animal; this reflects a several hundred fold increase in atrial size. Electrocardiograph analyses were performed in order to assess the effect of the atrial pathology on the cardiac conductance system. These studies indicate that there is a progressive increase in both the frequency and severity of irregularities in atrial depolarization accompanying the hyperplasia. Atrial tachycardia is evident, as well as an increase in heart rate, suggesting the presence of multi-focal atrial tachycardia. These abnormalities are eventually manifested as ventricular arrhythmias, and ultimately become severe enough to cause death.

A 002 IDENTIFICATION IN PORCINE BRAIN OF ANP-RELATED PEPTIDES, Hisayuki Matsuo, Department of Biochemistry, Miyazaki Medical College, Kiyotake, Miyazaki 889-16, JAPAN

Although atrial natriuretic peptide (ANP) has recently been verified to function as a neuropeptide in the central nervous system, its complete identification has not been done so far. We undertook the survey and isolation of ANP or its related peptides from porcine brain tissue, by using radioimmunoassay specific for α-ANP, coupled with an assay for relaxant effect on chick rectum. We have purified two peptides(I and II), showing ANP-like immunoreactivity, along with a peptide(III), eliciting potent rectum relaxant activity but no ANP-like immunoreactivity. By structural analyses, peptide I and II have been determined to be α-ANP(4-28) and α-ANP(5-28), respectively, indicating that proteolytic processing of ANP precursor in brain takes place in a manner different from that in heart. In addition, the amino acid sequence of peptide III will also be presented.

A 003 THE MOLECULAR BASIS FOR ATRIAL NATRIURETIC FACTOR GENE EXPRESSION C.E. Seidman, R. Fenton, R. Zeller, K.D. Bloch, R.T. Lee, D.W. Wong, and J.G. Seidman, Harvard Medical School, Boston, MA 02115

Expression of the gene encoding Atrial Natriuretic Factor (ANF) is stringently regulated throughout development in a tissue-specific fashion. We have studied the distribution of ANF mRNA during mouse cardiac embryogenesis using in situ hybridization. ANF mRNA was detected in a subpopulation of myocardial cells by gestational day 8. During day 9, abundant hybridization of the ANF probe to the atria and primitive ventricle was found. At day 14, high levels of ANF transcripts were detected in the atria and left ventricle (LV) whereas fewer cells in the right ventricle (RV) expressed the ANF gene. To assess if pathologic reexpression of the ANF gene was limited in the RV of adult animals, a subset of WKY rats that spontaneously develops biventricular hypertrophy (BVH) was studied. Normal WKY animals had low levels of LV ANF mRNA and undetectable RV ANF transcripts. BVH animals showed 6 fold greater LV ANF mRNA concentrations than WKY controls. RV mRNA levels in BVH animals were comparable to LV levels and dramatically greater that WKY RV controls. Spontaneous BVH animals demonstrate that both the LV and RV can concordantly respond to hypertrophy and reexpress the ANF gene at equivalent levels.

To study the molecular basis for tissue-specific and developmental regulation of ANF gene expression, we have analyzed cis-acting sequences using expression of the prokaryotic marker gene, chloramphenicol acetyl-transferase (CAT) as a functional assay. Hybrid ANF-CAT genes were introduced into primary cultured cardiocytes by electroporation. A 3.5 kb fragment containing sequences 5' of the ANF gene promoted significant CAT activity in atrial but not ventricular cardiocytes derived from one-day-old rats. Deletion analysis of this regulatory region demonstrated that 2.5 kb of 5' ANF sequences were required for high level atrial transcription, whereas hybrid genes containing less than 800 bp of sequence did not promote CAT activity. Cardiocytes derived from embryonic ventricles expressed the 3.5 kb ANF-CAT hybrid gene at levels comparable to atrial cells, suggesting that the nucleotide sequences controlling developmental regulation of ANF expression are contained in this 5' region. To further define the effects of this regulatory region in vivo, an ANF-CAT gene (containing 2.5 kb of 5' ANF sequences) was stably introduced into the mouse germline using a transgenic approach. Tissue-specific expression of CAT was demonstrated in transgenic animals, confirming that the regulatory region defined by in vitro assays is critical for appropriate ANF gene expression in intact animals.

Hormone Processing and Peptide Release

A 004 PROHORMONE PROCESSING AND ANF RELEASE MECHANISMS, Marc Cantin, Gaëtan Thibault, Jinfeng Ding, Helena Haile-Meskel, Raul Garcia, Mona Nemer, Jacques Drouin, Edyta Wrobel-Konrad and Jacques Genest, Clinical Research Institute of Montreal, 110 Pine Ave West, Montreal, Quebec, Canada, H2W 1R7.

In atria of rats, as determined by HPLC, amino acid sequencing and composition, only the propeptide of ANF (Asn 1-Tyr 126) is present in secretory granules. After stimulation by morphine or by blood volume expansion, two forms of ANF appear in large amounts in rat plasma: as determined by HPLC and sequencing, the C-terminal moiety of ANF (Ser 99-Tyr 126) and the N-terminal (Asn 1-Arg 98). Morphine and blood volume expansion produce a rapid decrease of atrial IR-ANF. At the E.M. level, both stimuli produce a rapid (within minutes) movement of secretory granules from the Golgi area to the subplasmalemma followed by exocytosis. Perfusion of the heart of rat or hamster with oxygenated Krebs fluid (Langendorff technique) yields both the C-terminal and the N-terminal of ANF in the effluent. An enzyme (IRCM-serine protease I) which is 9 times more abundant in atria than in ventricles generates three ANF fragments from the propeptide: (Ser 103-Tyr 126); (Arg 102-Tyr 126) and (Ser 99-Tyr 126). In man also, the C-terminal (Ser 99-Met 110-Tyr 126) and the N-terminal (Asn 1-Arg 98) are circulating as detected by RIA and by sequencing. The ventricular cardiocytes of rat, hamster and man contain low levels of ANF mRNA and IR-ANF which are increased by hypertrophy and in Brattleboro rats (without hypertrophy). In all these cases secretory granules appear in ventricular cardiocytes. Perfusion of the ventricles of control rats and hamsters and of cardiomyopathic hamsters again yields both peptides i.e. the C-terminal and the N-terminal moiety of IR-ANF. A high M.W. form of ANF which co-elutes with the N-terminal but is recognized by the C-terminal antibody also appears. Cultured rat ventricular cardiocytes also contain and secrete IR-ANF in amounts which decrease with the age of donor animals. In the impulse conducting system of the rat, IR-ANF is present in nodal and transitional cells of sino-atrial and atrio-ventricular nodes, in transitional cells of the right and left His bundle, in Purkinje cells (subendocardial and chordae tendinae spuriae (CTS)) and in "transition cells" located between Purkinje cells and working ventricular cardiocytes as determined by immunocytochemistry. In CTS of right and left ventricles, IR-ANF is 25 times more abundant per mg of protein than in the ventricular septum and, here again, of the high M.W. type as determined by HPLC. ANF mRNA is also present in CTS. While atria and possibly ventricles have an endocrine function, ANF is likely to have a paracrine effect on the impulse-conducting system.

A 005 CELLULAR MECHANISMS REGULATING ATRIOPEPTIN RELEASE, James E. Greenwald, Michael Apkon, Steven D. Sides and Philip Needleman. Department of Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

The mechanism of secretion of atriopeptin (AP) into the circulation is largely unknown. However, evidence suggests that elevations in atrial pressure (i.e., atrial stretch) may be the primary stimulus for release. Ventricular myocytes also synthesize and secrete AP, but whether ventricular secretion occurs by regulated and/or constitutive mechansims is currently unknown. Using a model of osmotic stress in isolated atrial and ventricular myocytes, we studied the effects of sarcolemmal stretch and the modulation of intracellular calcium $[Ca^{+2}]_i$ in the control of AP release. Atrial and ventricular myocyte suspensions were prepared from neonatal rats. Myocytes were subjected to osmotic stress for 15 minutes by resuspension in a physiologic electrolyte solution while mannitol was used to increase the osmolarity (200 to 310 mOsm). Myocytes exposed to the 200 mOsm solution demonstrated a five-fold increase in AP release when compared to cells exposed to 310 mOsm. Neonatal rat atrial and ventricular myocytes released AP as an inverse function of solution osmolarity at all osmolarities tested. Raising $[Ca^{+2}]_i$ by KCl depolarization (25 to 65meq/L) produced a voltage dependent release of AP. Myocytes were incubated with the Ca ionophore ionomycin (200 nM), the $[Ca^{+2}]_i$ chelator BAPTA AM (10 μM) or zero Ca media (1 mM EGTA) to modulate $[Ca^{+2}]_i$. Ionomycin inhibited basal and stimulated AP release by 19% and 52% respectively and AP release was potentiated by buffering $[Ca^{+2}]_i$ or extracellular Ca $[Ca^{+2}]_o$.

Experiment	1mM$[Ca]_o$	EGTA	BAPTA
300 mOsm	1	1.1±0.1	1.3±0.1
KCl 65 meq/L	2.2±0.1	3.0±0.4	4.4±0.3
200 mOsm	7.7±0.7	12.8±0.7	10.4±1.4

These data demonstrate atrial myocytes release AP in response to hypotonicity, a model of atrial stretch. Furthermore, ventricular myocytes release AP in response to stretch and depolarization, therefore demonstrating regulated mechanisms of secretion in these cell types. Analogous to renin secretion, AP release appears to be negatively modulated by $[Ca^{+2}]_i$.

A 006 SPECIFIC PROANF ACTIVATING ENZYME "ATRIOACTIVASE". T. Imada and T. Inagami, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232.

ANF is mainly synthesized in atrial myocytes and is stored in specific granules as pro-ANF with 126 amino acid residues (1). On the other hand the circulating form of ANF in rat plasma was shown to be a shorter peptide containing the 28 amino acid residue segment ANF(99-126) in the carboxyl terminal region of pro-ANF. These findings indicated that in rat atrium pro-ANF is converted to the circulating form during or just after secretion from the atrium. Recently we have obtained evidence for the presence of a pro-ANF processing enzyme in rat atria. In order to facilitate the specific detection of the enzyme, we used a synthetic substrate Boc-Ala-Gly-Pro-Arg-MCA (AGPR-MCA) which consists of the amino acid sequence on the amino terminal side of the peptide bond cleaved by the processing enzyme. The enzyme was found to be bound to the microsomal membrane fraction of rat atrial extract and was solubilized by 1.6 M KCl solution. In order to obtain a quantity of the enzyme to allow complete purification we used bovine atria. For the complete purification 5 successive column chromatography steps were needed. This enzyme was completely inhibited by p-aminobenzamidine, diisopropylfluorophosphate and leupeptin indicating that it is a serine protease. It was not inhibited by alkylating reagents or metal chelators indicating that this enzyme is not a sulfhydryl enzyme such as cathepsin B which is commonly found in the cytosolic fraction of atriocytes or an metalloprotease. The molecular weight obtained by gel filtration was 580,000 but by SDS-gel electrophoresis gave 6 bands with molecular weights of 26,000, 26,500, 29,000, 31,000, and 31,500. The localization of the enzyme in microsomal fraction in the homogenate suggests that it is bound to membranes or ANF on the containing granule. The most important feature of this enzyme is its specificity with respect to its products and its subcellular localization. It cleaves exclusively the Arg_{98}-Ser_{99} peptide bond of proANF (ANF_{1-126}) isolated from rat atrium and produces only ANF_{99-126} containing 28 amino acid residues as shown in Fig. 1. It does not cleave other arginyl peptide bond such as Arg_{101}-Arg_{102}-Arg_{103}. Therefore the product of the aciton of this enzyme is exclusively $ANF_{(99-126)}$ with 28 amino acid residues, the naturally circulating form. This property makes the enzyme the prime candidate for the proANF activating enzyme. It is clear that the atrial specific activating enzyme is different from other enzymes specific for pituitary or pancreatic hormones.

Receptors

A 007 DEMOSTRATION OF TWO TYPES OF RECEPTORS FOR ATRIAL NATRIURETIC FACTOR BY SIMULTANEOUS PURIFICATION FROM PLASMA MEMBRANES OF BOVINE ADRENAL CORTEX. T. Inagami, T. Takayanagi, R. Snajdar, K.N. Pandey, T. Imada, K.S. Misono. Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232.

Crosslinking or photoaffinity labeling of receptors in various tissues revealed the presence of ANF receptor with apparent molecular weight of 65,000-70,000, 90,000, 125,000 - 140,000 and 180,000. ANF had been shown to increase cGMP production of various target organs by stimulating the membrane bound form of guanylate cyclase. However, cGMP cannot explain the inhibition of aldosterone release from ANF. These observations raised the question as to the relationship of the roles of the multiple types of receptors in mediating a variety of action by ANF and the possible existence of two different pathways of intracellular signal transduction mechanism of ANF action, one mediated by cGMP and the other without the intermediacy of cGMP. To clarify possible multiple pathways of ANF action, we have devised methods to stabilize and purify the ANF receptors and to characterize these receptors. ANF receptor of bovine adrenal cortex was solubilized with Triton X-100 and purified by sequential chromatography on ANF-agarose, GTP-agarose, and wheat germ agglutinin-Sepharose. Two subtypes of ANF receptors were islated, both of which showed specific ANF binding, whereas one of the ANF receptor subtypes also possessed significant cyclase activity. Both of the receptors showed high capacities (Bmax=5.7-6.8 nmol/mg of protein) and high afinities (Kd=5.7-6.8 pM) for high affinity (Ki=150-220 pM) to C-terminal tuncated ANF analogs, whereas the cyclase-containing receptor had a much weaker affinity (Ki = 10^6-10^7 pM). When treated with dithiothreitol, the purified cyclase-containing and cyclase-free ANF receptors migrated as a single band at Mr 135,000 and 62,000, respectively, in SDS-PAGE. The purified cyclase free recpetor is not a product derived from the cyclase-containing receptor because (i) two proteins with Mr of 135,000 and 62,000 were specifically labeled with 4-azidobenzoyl ^{125}I-ANF-(102-126) in intact membranes; (ii) the truncated ANF analogs (10^4 pM) prevented the photolabeling of the 62,000-dalton protein; and (iii) two-dimensional peptide mapping showed more than 90% difference between the profiles of the two purified ANF receptor subtypes. This study provides first direct evidence for the existence of two distinct ANF receptors which are different not only in their pharmacolcgical properties but also in their primary structure.

A 008 PHARMACOLOGICAL AND BIOCHEMICAL STUDIES OF NOVEL ATRIAL NATRIURETIC PEPTIDE CLEARANCE RECEPTORS, J. Lewicki, J.G. Porter, L. Gregory, D. Schenk, F. Fuller, A. Arfsten, G. McEnroe, T. Maack, and R. Scarborough, California Biotechnology Inc. Mountain View, CA 94043 and Cornell University Medical Center New York, NY 10021.

We have reported recently that a novel atrial natriuretic peptide (ANP) receptor subpopulation mediates the sequestration and metabolic clearance of circulating ANP (Maack, T., et al., Science, in press). Structure-activity analysis has revealed that these receptors, termed C-ANP receptors, exhibit an absolute specificity for ANP sequences. However, C-ANP receptors bind a variety of truncated, deleted, D-amino acid substituted and linearized ANP fragments with high affinity (Ki=50-1000 pM). The insensitivity of C-ANP receptors to drastic structural changes of the ANP molecule is unprecedented for peptide hormones which transduce biological signals and is consistent with the proposed clearance function of this site.

To explore the detailed mechanisms underlying C-ANP receptor-mediated clearance of ANP, pharmacological, biochemical and molecular biological approaches have been employed. We have determined that acute or chronic administration of C-ANP specific ligands, such as [des (18-22)] rANP (4-23)-NH_2 (C-ANP (4-23)), leads to a significant natriuresis and diuresis in several distinct animal models. These C-ANP (4-23)-induced changes in renal function are delayed in onset and substantially prolonged in duration relative to the natriuresis and diuresis induced by ANP (1-28). This biological response profile results from the saturation of C-ANP receptors by C-ANP (4-23) leading to a decreased metabolic clearance and a marked increase in plasma levels of endogenous ANP (1-28).

Complementary DNA sequences encoding the C-ANP receptor have been cloned successfully from BASM and human kidney libraries. These cDNAs encode a highly conserved receptor comprised of an extracellular domain (436 amino acids), a transmembrane domain (23 amino acids) and a short cytoplasmic tail (37 amino acids). Interestingly, the finding of a single transmembrane domain proximal to a short, carboxyl-terminal cytoplasmic tail is consistent with the general structure of other putative clearance receptors. At present, the C-ANP receptor has been expressed in a variety of mammalian cells using a recombinant vaccinia virus (VV) vector. The recombinant C-ANP receptor binds a range of ANP analogues in a manner indistinguishable from native receptor. The same VV vector containing C-ANP receptor sequences has been used to inoculate rabbits, and specific antibodies, which block ANP association with the C-ANP receptor, have been prepared. These antibodies, cloned DNA sequences, and receptor selective ligands will be invaluable in addressing a variety of issues pertaining to the role of these novel receptors in the actions of ANP.

Endocrine Interactions and Intracellular Mediators

A 009 NEGATIVE REGULATION OF ATRIAL NATRIURETIC FACTOR RECEPTOR COUPLED MEMBRANE GUANYLATE CYCLASE BY PHORBOL ESTER: POTENTIAL KINASE-C REGULATION OF CYCLIC GMP SIGNAL, Rama Kant Jaiswal and Rameshwar K. Sharma, Department of Physiology & Biophysics, University of Tennessee, Memphis, 894 Union Avenue, Memphis, TN 38163.

Isolated adrenocortical carcinoma cells of rat possess a high density of atrial natriuretic factor (ANF) receptors which are coupled with membrane guanylate cyclase. Both guanylate cyclase and ANF receptor activities appear to reside on a single 180-kD protein as evidenced by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), Western blot analysis of the crude enzyme, stoichiometric binding of ANF and by isoelectric focusing (1). The enzyme appears to be ubiquitous and conserved, since Western blot analysis indicates its presence in rat adrenal and testis, in mouse testis, in bovine aortic endothelial and outer rod membranes of the eye. Similarly, immunoblot analysis indicates the 180-kD membrane guanylate cyclase antibody cross-reactivity with rat kidney-glomerulus, papillary and endothelial cells and as diverse tissues as chicken retina and spisula sperms and eggs. As a first step to elucidate the molecular mechanisms of regulation of this enzyme in transmembrane signaling, we have investigated the interaction of ANF-dependent membrane guanylate cyclase with protein kinase C signal.

Pretreatment of rat adrenocortical carcinoma cells with phorbol 12-myristate 13-acetate (PMA), a known activator of protein kinase C, attenuated the ANF-stimulated cyclic GMP accumulation in a dose dependent manner. The half maximum inhibitory concentration of PMA was 10^{-10}M. The inactive phorbol ester alalogs - phorbol 12, 13 didecanoate and 4β-phorbol, did not inhibit the hormonal dependent cyclic GMP accumulation. When these cells were incubated with PMA in the presence of 1-(5-Isoquinolinyl-sulfonyl)-2-methyl piperazine, a protein kinase C inhibitor, the PMA mediated attenuation of ANF-stimulated cyclic GMP formation was blocked. These results suggest that protein kinase C negatively regulates the ANF-receptor coupled membrane guanylate cyclase system in these cells.

Analogous to the situation with phorbol ester receptor, vasopressin receptor signal also negatively regulates the ANF-depenent formation of cyclic GMP and positively regulates the phosphatidylinositol turnover. The link between vasopressin and ANF receptor signals might also be through protein kinase C. The α_1-adrenergic signal transduction positively regulates membrane guanylate cyclase and negatively regulates the adenylate cyclase. Together, the results indicate that these transmembrane receptor signals in which cyclic GMP plays a bona fide second messenger role are intertwined.

1. Paul, A.K., Marala, R.B., Jaiswal, R.K., & Sharma, R.K. (1987) Science 235, 1224-1226.

A 010 ROLE OF ACTIVATION OF PARTICULATE GUANYLATE CYCLASE IN THE EXPRESSION OF BIOLOGICAL ACTIONS OF ANF, Pavel Hamet and Johanne Tremblay, Clinical Research Institute of Montreal 110 Pine Ave. W. Montreal Canada H2W 1R7. Cyclic GMP egress into plasma and urine as a faithful biological marker of the action of ANF. The extracellular secretion of cGMP has been studied in cultured cells where it competes with cAMP for the same pathway. One of the characteristics of particulate guanylate cyclase is its apparent persitent stimulation, reflected *in vivo* by prolonged increases of extracellular cGMP, correlating better with natriuresis than the plasma levels of ANF. After chronic infusion of ANF, although a persitent effect is still seen on the decrease of blood pressure, there is no increase of plasma or urinary cGMP. *In vitro* studies suggest that plasma cGMP originates from smooth muscle cells and endothelial cells, yet after ANF stimulation, mainly the endothelial cells are responsible for the plasma increases of cGMP. Studies with Met-O-ANF helped to dissociate diuretic and natriuretic effects of ANF. Free water clearance is indeed stimulated by parent and oxidized compounds, the osmotic clearance being stimulated only by the native compound. Both osmotic and free water clearances are inhibited by the partial antagonistic effect of Met-O-ANF. The relevance of the particulate guanylate cyclase stimulation in ANF function is illustrated by the correlation of increases of cGMP in plasma and urine with diuretis and natriuresis as induced by recumbent posture, permitting the assessment of the biological effectiveness of endogenous ANF.

A 011 ANF RECEPTORS AND THE GUANYLATE CYCLASE - CYCLIC GMP SYSTEM, Ferid Murad, Stanford University and Veterans Administration Medical Center, Palo Alto CA 94305.
The increased synthesis of cyclic GMP from GTP by the soluble or particulate isoenzyme forms of guanylate cyclase mediates the vasodilatory effects of three classes of vasodilators: the nitrovasodilators, the endothelium-dependent vasodilators and ANF. The soluble and membrane-associated isoenzyme forms of guanylate cyclase have different kinetic, physiochemical and antigenic properties and primary structures. The direct acting nitrovasodilators such as nitroprusside, nitroglycerin and other agents that form the reactive nitric oxide free radical activate the soluble isoenzyme, increase cyclic GMP-dependent protein kinase activity, and alter the phosphorylation of myosin light chain. The endothelium-dependent vasodilators such as acetylcholine, histamine, A23187, ATP, thrombin, etc., which lead to the formation of endothelial-derived relaxant factor (EDRF) also activate soluble guanylate cyclase. The cascade of events distal to cyclic GMP synthesis appears to be identical. A third class of vasodilators, ANF, however, specifically activates the particulate isoenzyme form of guanylate cyclase and increases cyclic GMP synthesis and cyclic GMP-dependent protein kinase activity. The effects of ANF are endothelium-independent and are mediated through a subpopulation of receptors that are coupled to particulate guanylate cyclase that we have designated ANF-1. This subclass of ANF receptors (ANF-1) copurifies with particulate guanylate cyclase and is 130,000 daltons suggesting that these activities reside in the same transmembrane glycoprotein. Some forms of particulate guanylate cyclase, however, are not associated with ANF binding and activation. Most tissues and cells possess a second ANF binding site of 66,000 daltons that we have designated ANF-2 sites. These latter sites generally represent 90 to 98% of total ANF binding and probably exist as a homodimer with subunits joined through a disulfide bond. The second messenger coupled to the occupancy of the ANF-2 site and its physiological functions are not known. To date, most observations support our hypothesis that the vasodilatory effects of ANF are mediated through increased cyclic GMP synthesis. However, ANF can also activate particulate guanylate cyclase and increase cyclic GMP synthesis in numerous and diverse tissues and cells such as liver, testes, endothelial, epithelial and glial cells, fibroblasts, etc. These effects suggest that ANF probably has important physiologic and metabolic effects in addition to the cardiovascular, renal and adrenal effects. While it is not known which of the numerous effects of ANF are mediated through cyclic GMP, their interrelationships have provided important clues to the actions of these agents.

Renal Response and Mechanisms

A 012 FACTORS DETERMINING THE NATRIURETIC RESPONSE TO ATRIOPEPTIN (AP), Edward H. Blaine, Dale A. Hartupee, Angelo J. Trapani and John P. Koepke, G.D. Searle R&D, Washington University School of Medicine, St. Louis, MO 63110.

The mechanism by which AP enhances urinary Na excretion is unknown. The possibilities include direct inhibition of tubular reabsorption, enhancement of glomerular filtration rate (GFR), and changes in intrarenal hemodynamics, especially vasa recta flow. Maintaining renal arterial pressure constant with angiotensin II or methoxamine during intravenous AP infusion greatly potentiates the natriuretic response[1]. Arginine vasopressin (AVP) at nonpressor doses can also potentiate AP-induced natriuresis[2]. AVP could augment AP-induced natriuresis either by an intrarenal or systemic mechanism. To delineate between these two possibilities, we compared the effects of renal artery (ira, 0.12 mU/kg·min) and intravenous (IV, 1.2 mU/kg·min) infusions of AVP on the natriuretic response to AP (IV, 0.36 nmol/kg·min) in two groups of anesthetized dogs. Na excretion was increased from 20±8 to 46±16 uEq/min by AP alone and was further increased to 301±75 uEq/min by IV AVP (n=5). On the other hand, ira AVP did not affect the increase in Na excretion caused by AP alone (n=3, basal 21±8, AP alone 101± 15, AP and ira AVP 103±28 uEq/min). We concluded that the potentiation of AP-induced natriuresis by IV AVP was extrarenal in origin. Bishop's laboratory has demonstrated that AVP inhibits renal nerve activity before significantly increasing arterial pressure[3]. Could this be the mechanism of the enhanced natriuresis? To test this hypothesis that potentiation of AP-induced natriuresis by IV AVP is mediated via the renal nerves, we denervated one kidney of anesthetized dogs prior to AP infusion (n=5). The innervated kidney showed typical potentiation of the AP-induced natriuresis with IV AVP infusion. The denervated kidney showed an exaggerated natriuresis to AP alone with no further increase in Na excretion during IV AVP infusion (Table).

	Urinary Na Excretion (uEq/min)		
	Basal	AP Alone	AP & AVP
Innervated	35±17	146±49	213±47
Denervated	33± 5	302±38	265±40

No differences were observed in GFR between the kidneys. Thus, inhibition of renal nerve activity is a likely explanation for the potentiation of AP-induced natriuresis by AVP. How the renal nerves act to affect Na excretion in this circumstance is yet to be determined.

1. Marsh et al., Abst. 67th Ann. Mtg. Endocrine Soc., 1985, p221. 2. Blaine and Nicolodi, Abst. First World Congress on Biol. Active Peptides, 1986. 3. Undesser et al., Circ. Res. 56:410, 1985.

A 013 THE RELEASE OF ATRIAL NATRIURETIC PEPTIDES AND ITS ROLE IN VOLUME REGULATION

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The role of endogenous atrial natriuretic peptides (ANP) in volume regulation was investigated with a long-acting monoclonal antibody. This antibody specifically blocks the effects of ANP in vivo without affecting baseline diuresis and natriuresis.

Acute volume expansion was induced by injection of 15 or 20 ml/kg b.w. of saline or whole blood in rats (conscious or thiobabitone-anaesthetized). The release of endogenous ANP mediates the initial natriuretic and diuretic response to isotonic volume expansion and reduces the increase in central venous pressure. Under certain conditions, however, the participation of other mechanisms in acute volume regulation can also be detected: The sustained diuresis caused by volume expansion with fluids containing albumine is only partially blocked by the monoclonal antibody, and in response to a high intravenous sodium load the urinary sodium concentration can be raised independently of the ANP system.

Chronic volume expansion was induced by treatment of normotonic rats with the sodium-retaining vasodilatator minoxidil. Sodium excretion normalized after 1 day of treatment in fed rats, but ANP plasma levels remained increased by 100-200%. Administration of the monoclonal antibody after 2 days of minoxidil treatment did not reduce sodium excretion. Chronic renal failure due to renal ablation (5/6 nephrectomy) was used as another model of chronically increased ANP plasma levels. Diuresis was increased dramatically after 5/6 nephrectomy but this increase was not blocked by the monoclonal antibody. It may be concluded that endogenous ANP release in this polyuric model of chronic renal failure does not account for the observed diuresis. On the other hand, exogenous ANP still elicits a renal response in 5/6 nephrectomized rats even when their glomerular filtration rate is severely reduced.

A 014 RENAL RECEPTORS AND MECHANISMS OF ACTION OF ATRIAL NATRIURETIC FACTOR: PHYSIOLOGICAL CHARACTERIZATION AND ROLE OF CLEARANCE (C-ANF) RECEPTORS, T. Maack, D.R. Nussenzveig, M. Suzuki, R.M. Scarborough, J.A. Lewicki and F.A. Almeida. Dept. of Physiology, Cornell University Medical College, New York NY 10021 and California Biotechnology Inc., Mountain View CA 94043.

There are two functionally distinct classes of ANF receptors in kidney and vascular tissues: B-ANF receptors mediate its known renal effects and increase in cGMP, whereas, C-ANF receptors are biologically silent and serve as clearance binding sites for the hormone (Maack et al. Science, 1987, In Press). In isolated perfused rat kidney (IK), isolated glomeruli (G) and cultured mesangial cells (M), biologically active ANF1-28 binds with similar high affinity (Kd, 50-150pM) to B-ANF and C-ANF receptors, whereas, des[Q18S19G20L21G22]ANF4-23-NH2 (C-ANF4-23) binds only to C-ANF receptors. Using this analog, we determined that C-ANF receptors constitute either the overwhelming majority (>95% in IK; 80% in G) or an important fraction (40% in M) of the total ANF receptor population in these preparations. In IK, there is a remarkable similarity between ANF1-28 binding curves in kidney cortex and dose-response curves of all its known renal vascular, hemodynamic and excretory effects. On the other hand, C-ANF4-23 does not have effects on its own and does not inhibit any of the known effects of ANF1-28 in IK or the ANF1-28 induced increase in cGMP in M. In anesthetized or conscious rats C-ANF4-23 (1 μg/min/Kg body wt) increases plasma levels of endogenous irANF by app. threefold and this increase accounts entirely for the natriuretic and blood pressure lowering effects of the analog in intact rats. At this dose C-ANF 4-23 decreases volume of distribution(Vd) and metabolic clearance rate(MCR) of [125I]-ANF1-28 in half. A tenfold higher dose of C-ANF4-23 further decreases Vd and MCR to 1/3 and 1/4 of their control values, respectively. Thus, binding to C-ANF receptors accounts for app. 2/3 of the Vd and MCR of ANF in the rat. C-ANF receptors also mediate app. 2/3 of the total hydrolysis of ANF in the rat, as estimated by the appearance of labeled hydrolytic products in plasma after the administration of [125I]-ANF1-28 in absence or presence of C-ANF4-23. Structural requirements of atrial peptide analogs for binding to C-ANF receptors are far less stringent than those for binding to B-ANF receptors. Thus, analogs that bind to C-ANF but not to B-ANF receptors may be therapeutically useful since they increase plasma levels of endogenous ANF by decreasing its metabolic clearance rate. The findings demonstrate that C-ANF receptors constitute the majority of renal and systemic receptors of ANF and that these receptors are mainly responsible for the very large volume of distribution, high metabolic clearance rate and extensive hydrolysis of ANF in the rat. In this manner, C-ANF receptors are likely to have an important role in the modulation of plasma levels of ANF.

A 015 THE MEDULLARY COLLECTING DUCT IN ANF NATRIURESIS, H. Sonnenberg, Dept of Physiology, Univ. of Toronto, Canada M5S 1A8.

Early micropuncture studies implicated the medullary collecting duct as the site of sodium transport inhibition due to atrial natriuretic factor (ANF). With the recognition of the renal hemodynamic effects of ANF, changes in GFR and medullary blood flow and consequent "medullary washout" were postulated as effectors of ANF natriuresis. Briefly, reduction of ascending limb sodium reabsorption was thought to overwhelm downstream transport capacity, leading to 'spillover' of the ion into the urine. However, recently we showed conclusively that ANF natriuresis is associated with specific inhibition of Na transport in the medullary collecting duct system. The quantitative effect of this inhibition may vary, depending on other regulatory factors. Rats fed a low-salt diet have increased duct sodium reabsorption compared to those on high-salt. Superimposition of the inhibitory effect of ANF leads to a larger natriuresis in the latter group. In vitro preparations of duct cells indicate that ANF acts via inhibition of an amiloride-sensitive sodium channel. However, in vivo, although amiloride indeed reduced net transport at this site, the effect of ANF was additive, suggesting that inhibition of the sodium channel is not the only mechanism of ANF natriuresis.

Cardiovascular I

A 016 Relative effects of atrial peptides and atrial stretch receptors on hemodynamics and renal function. Kenneth L. Goetz. Division of Experimental Medicine, St. Luke's Hospital and Foundation, Kansas City, MO 64111.

The well-known cardiovascular and renal effects elicited by atrial distension conceivably could be mediated by neural reflexes elicited by atrial stretch receptors or by the release of atrial peptides. This presentation will focus on experiments designed to evaluate the relative potencies of these two atrial mechanisms. Earlier experiments designed to compare the effects of atrial stretch with the effects of pharmacological doses of atriopeptin demonstrated that the hemodynamic responses elicited by these two stimuli were quite different. On the other hand, renal responses induced by these two stimuli were similar, a finding consistent with the hypothesis that the increase in urine flow and sodium excretion elicited by atrial distension is mediated by the associated release of atrial peptides. However, later experiments demonstrated that cardiac denervation prevents the diuretic and natriuretic responses induced by atrial distension even though circulating atrial peptides increase normally. These results implied that neural reflexes arising from atrial stretch receptors were responsible for the renal changes elicited during atrial distension. Other evidence indicating that atrial peptides have relatively little effect on renal sodium excretion was derived from experiments in which plasma atriopeptin levels were increased 3-, 7-, and 11-fold by intravenous infusions of α-human atrial peptide to conscious dogs. Atriopeptin caused a delayed natriuresis that was less marked than that elicited by previous atrial distension experiments even though the two higher infusion rates increased plasma atriopeptin to higher concentrations than those measured during atrial distension.

The relative effects of atrial peptides and atrial reflex mechanisms on the renal response elicited by atrial distension were also assessed by infusing α-human atrial peptide into conscious dogs during a 40 minute control period. The infusion then was stopped abruptly and left atrial pressure was increased 8 mmHg by inflating a balloon positioned above the mitral valve. Although plasma atrial peptide levels decreased substantially during the 40 minute period of atrial stretch, urine flow and sodium excretion increased during this time. Similar results were obtained when intravascular volume expansion was substituted for atrial stretch. The results of these experiments imply that circulating levels of atrial peptide do not play a dominant role in influencing sodium excretion either during atrial distension or in response to saline infusion.

A 017 CARDIOVASCULAR ACTIONS OF ATRIOPEPTINS. T.H. Hintze, M. Patel, J.T. Shapiro, D.J. O'Dea, G.C. Shapiro, H. Stern and A. Loud. Department of Physiology and Pathology, New York Medical College, Valhalla, New York 10595.

Atrial stretch induces atriopeptin release and this has been correlated with changes in mean atrial pressure. However, atrial filling and hence passive stretch of the atrium occurs only during the "v" wave of the atrial cycle. In order to better understand the physiologic stimulus for atriopeptin release we measured a and v wave atrial appendage wall stress during changes in heart rate and volume expansion. Atriopeptins were preferentially located in the atrial appendage. Left atrial appendage atriopeptin content (1020 ± 240mg/gm) was significantly higher than left atrial body (470 ± 130ng/gm) and significantly higher than left venticuler (89 ± 25 ng/gm). By fixing the atria in situ and then in KCl (D) or $BaCl_2$ (S) solutions and using quantitative morphometric techniques, we derived atrial wall thickness constants during diastole (D) and systole (S). Left atrial appendage wall thickness was 2.40 ± 0.20 and 2.88 ± 0.17 mm during D and S. Atrial pacing at 180 b/min while measuring atrial pressure and dimensions in chronically instrumented conscious dogs showed that "a" wave and "v" wave pressure and dimensions fell whereas plasma ANF increased 90 ± 10%. At 240 b/min a paradoxical increase in "a" wave pressure and diameter occurred along with a rise in "v" wave pressure and diameter. Calculated wall stress increased during the a wave 310 ± 45% whereas during the v wave wall stress increased 97 ± 15% with no further increase in ANF. During volume expansion of 500 and 1000mls "a" and "v" wave wall stress increased 138 ± 5% and 385 ± 14% respectively. When multiplying by heart rate i.e. minute wall stress, "v" wave minute wall stress increased to a significantly greater degree than "a" wave ($p<0.05$). Therefore, in chronically instrumented conscious dogs release of atriopeptin is related to "v" wave or atrial filling, than to "a" wave or atrial contraction. Since mean atrial pressure is integral of both atrial contraction, a wave, and atrial filling, v wave, it may be an inappropriate index of atrial peptide secretion.

A 018 FACTORS INFLUENCING HEMODYNAMIC RESPONSES TO ATRIAL NATRIURETIC FACTOR IN VIVO, Rodney W. Lappe and Joy A. Todt, Wyeth Labs., Inc., Philadelphia, PA 19101.

Atrial natriuretic factor (ANF) is a potent vasorelaxant of isolated vascular smooth muscle in vitro. Unfortunately the in vitro vascular effects of ANF do not always translate to vasodilator responses in conscious animals. Many factors appear to modify the hemodynamic effects of ANF. Infusion of ANF in spontaneous hypertensive (SHR) or normotensive rats reduces mean arterial pressure, but increases regional vascular resistance in the renal, mesenteric and hindquarter vascular beds. Antagonism of sympathetic function by chemical or surgical sypathectomy attenuates the regional vasoconstrictor responses to ANF in the rat. Similar results are observed with the nitrovasodilator nitroprusside. These data indicate that ANF is probably not a direct-acting vasoconstrictor agent. Rather it would appear that the regional vasoconstriction during ANF infusion is due to compensatory increases in sympathetic vasoconstrictor tone.

The hemodynamic effects of ANF are also markedly influenced by resting arterial pressure and vascular tone. Infusion of ANF into several models of conscious hypertensive rats elicits enhanced reductions in mean arterial pressure. Steroid(DOCA)-salt hypertensive rats are the most sensitive to the hypotensive effects of ANF followed by renal (renin dependent) hypertensive rats. No regional vasodilation accompanies the hypotensive actions of ANF in the DOCA-salt rats. The increased sensitivity to ANF in the DOCA rats is not observed early during the onset of DOCA-salt hypertension (2 weeks after implant of DOCA pellet), but is manifested during the maintenance phase of hypertension. Various vasoconstrictor agents also appear to be more susceptible to the actions of ANF. The hypotensive responses to ANF are enhanced in rats made acutely hypertensive with equi-pressor infusions of norepinephrine, angiotensin II or neuropeptide Y. The effects of ANF are similar in the norepinephrine and angiotensin II-treated groups of rats. However the potency and efficacy of the atrial peptide is markedly enhanced in the neuropeptide Y-treated rats. These data demonstrate that the hypotensive actions of ANF are influenced not only by increases in basal vascular tone, but also by various types of vasoconstrictor agents.

Cardiovascular II

A 019 CONTRASTING ACTIONS OF ATRIAL NATRIURETIC PEPTIDE ON THE CARDIOVASCULAR SYSTEM. B.A. Scoggins, D.G. Parkes, J.G. McDougall and J.P. Coghlan. Howard Florey Institute of Experimental Physiology and Medicine, Parkville, 3052, Australia.

Studies in many animal species including man have established that atrial natriuretic peptide (ANP) has significant effects on the cardiovascular system. There is, however, considerable variability in the pattern and extent to which hemodynamic parameters are changed. While part of these differences in response are probably related to the species, to the peptide and to its rate of administration, studies in conscious sheep have shown that the duration of infusion is an important determinant of the observed response.

Acute administration of h ANP (1-28) (20 μg by iv injection) produces a rapid fall in mean arterial pressure (MAP) from 65 ± 1 to 57 ± 2 mmHg. This fall in MAP was associated with a rise in cardiac output (CO) and heart rate (HR) and a fall in total peripheral resistance (TPR). After 5 min MAP, HR and TPR were normal but CO fell progressively for the next 10 min. Similar findings of an immediate vasodilator action of ANP have been reported by others in dogs and rats.

Infusion of ANP (1-28) at 20-50 μg/h iv into sheep for 60 min produced a dose dependent fall in stroke volume, and CO and an increase in HR and TPR. Resultant falls in MAP were small (2-5 mmHg). Blood volume (SV) decreased and an increase in the gain of the heart rate-blood pressure reflex was observed. The reflex increased in heart rate and TPR which occur with ANP can be abolished by autonomic blockage and attenuated by ACTH treatment or volume expansion.

In contrast, infusion of ANP at 20 μg/h for 5 days in sheep results, after 24 hr, in significantly greater falls in MAP (10-15 mmHg) which are entirely due to a fall in TPR. CO, HR and BV initially changed have returned to normal.

ANP at 20 μg/hr in conscious sheep has no natriuretic activity suggesting the observed effects on the cardiovascular system are not secondary to the renal action of the peptide.

In summary, ANP produced an initial vasodilation which is rapidly reversed by reflex increases in TPR, resulting from the effects of the peptide on MAP and CO. The effects of ANP on SV and CO may be due to effects on the venous circulation. However, withing 24 hours the reflex responses are attenuated and the vasodilator effects of the peptide result in a progressive fall in MAP. These results may explain the variability in the responses to ANP observed by others.

Cardiovascular I

A 020 IMMEDIATE AND PROLONGED HEMODYNAMIC ACTIONS OF ATRIAL NATRIURETIC FACTOR

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Atrial natriuretic factor (ANF) decreases cardiac output at pharmacologic doses. This is not initiated by direct myocardial depression, but by a peripheral vascular action of ANF resulting in diminished venous return and lower central venous pressure (CVP). ANF is often suggested as dilating veins and increasing circulatory capacitance. However, total circulatory capacitance, determined by mean circulatory filling pressure and blood volume, was not increased in rats. ANF reportedly did not dilate hand veins in humans nor increase venous compliance in dogs. Also, relative to other vasodilators such as nitroglycerin (NG), ANF has little action on veins in vitro. Thus, there is no evidence that ANF exerts its hemodynamic effects by venodilation. We compared the circulatory action of ANF and NG in conscious rats with heart failure due to coronary ligation 3 wk prior to study. ANF or NG was infused at 0.1, 0.25, 0.5 or 2, 5, 10 ug/min/kg, respectively for 20 min each dose. CVP decreased ($P<0.05$) during all doses in both groups with decreases (ANF) or no change (NG) in cardiac output, suggesting diminished venous return. There were striking differences between groups in hematocrit (Hct) changes. NG decreased ($P<0.05$) Hct during all doses, suggesting immediate enhanced capillary absorption. ANF increased ($P<0.05$) Hct during the two higher doses, suggesting delayed decreased blood volume. Enhanced capillary absorption is consistent with NG's venodilatory activity resulting in reduced postcapillary resistance. The opposite effect of ANF on Hct indicates a mechanism other than venodilation. The delayed change in Hct but immediate lowering of CVP by ANF indicates an action other than decreased blood volume initiated the diminished venous return. We propose that ANF indirectly increases postcapillary resistance and, hence, resistance to venous return.

To examine the prolonged effects of ANF, spontaneously hypertensive rats were implanted with arterial catheters, and osmotic pumps delivering ANF intravenously at 2 pmol/min/kg or mock pumps (controls). After 6 days, mean arterial pressure, measured directly in the conscious, unrestrained rats, was 160 ± 4 and 175 ± 5 mmHg ($P<0.05$) in the infused and control groups, respectively; plasma immunoreactive ANF was 157 ± 34 and 144 ± 11 pg/ml. Prolonged ANF also decreased heart rate (319 ± 12 vs 360 ± 9 b/min, $P<0.05$).

In conclusion, an immediate action of ANF in pharmacologic doses seems to be increased postcapillary resistance. The antihypertensive effect of prolonged infused ANF at a dose causing minimal change in plasma ANF is associated with a lower heart rate, suggesting altered autonomic control of the heart.

Presence and Function of Atrial Peptides in the Nervous System

A 021 ATRIAL NATRIURETIC PEPTIDE IN THE CENTRAL NERVOUS SYSTEM AND ITS POSSIBLE FUNCTION, Hiroo Imura and Kazuwa Nakao, Dept of Medicine, Kyoto University Faculty of Medicine, Kyoto 606, Japan.

The presence of atrial natriuretic peptide (ANP) in the brain was demonstrated by RIA and immunohistochemistry. We studied molecular forms of ANP in the brain and its possible function. We also studied the relationship between the brain and cardiac ANP secretion. Using RIA for α-ANP (ANP 99-126) and N-terminal peptide (ANP 1-25) combined with HPLC, we demonstrated that major molecular forms in the rat brain are ANP 102-126 and ANP 103-126 but not ANP 99-126. The precursor form (γ-ANP) and the N-terminal fragment was also detected. These results indicated that Arg-Arg at positions of 101 and 102 of ANP precursor is the processing site in the brain different from a single Arg at the position of 98 in the heart. Since α-ANP-like immunoreactivity is distributed abundantly in the AV3V region and in hypothalamic nuclei, we studied the effect of intracerebroventricular (icv) injection of α-ANP or its analogues on drinking, salt appetite, blood pressure, vasopressin and ACTH secretion in rats. Angiotensin II (AII) induced drinking, blood pressure rise, vasopressin and ACTH secretion were all counteracted by icv injection of α-ANP. α-ANP also inhibited salt intake in SHR. Since anti-α-ANP antisera given icv significantly augmented drinking, the above-mentioned effects of ANP may have of physiological significance.

Icv injection of AII did not augment ANP secretion from the heart but significantly enhanced volume expansion-induced ANP secretion. This effect was blunted by V_1 antagonist for vasopressin and α-adrenergic antagonists suggesting that vasopressin and central α-adrenergic mechanism are involved in this mechanism. Icv injection of ANP also blunted AII effect on cardiact ANP secretion. These results suggest that the brain is involved in regulating ANP secretion from the heart.

It is concluded that the brain ANP system seems to play important roles in regulating fluid and blood pressure homeostasis.

A 022 HYPOTHALAMIC ACTIONS OF THE ATRIAL PEPTIDES TO ALTER HORMONE SECRETION FROM BOTH THE ANTERIOR AND POSTERIOR PITUITARY, Willis K. Samson, Department of Physiology, UTHSC, Dallas, Texas 75235.

The presence of atrial peptide (AP) binding sites and immunoreactivity in hypothalamic sites known to be involved in the control of anterior and posterior pituitary function suggests a role for the peptides in neuroendocrine physiology. Indeed the diuretic and natriuretic actions of AP in the kidney are complimented by the ability of the peptide to act within the brain to inhibit vasopressin (AVP) secretion, water drinking and salt preference. The locus of action to inhibit AVP secretion has been identified to be primarily within the hypothalamus. In vivo and in vitro studies have suggested this action of AP to be due to an antagonism of the stimulatory effect of angiotensin II. These actions of AP within the brain reinforce its peripheral actions to control fluid and electrolyte homeostasis.

Recently attention has been focused on the possible role AP plays in anterior pituitary function. AP immunoreactivity can be detected in the median eminence and in discrete hypothalamic sites, suggesting either a releasing factor action in the pituitary or a neuromodulatory effect centrally to alter hypothalamic control of the gland. We and the St. Louis group have demonstrated the ability of i.v. AP infusion to inhibit luteinizing hormone (LH) secretion. This effect is not exerted at the level of the anterior pituitary gland since neither the in vivo nor in vitro response to LH-releasing hormone (LHRH) challenge is altered by AP exposure and central administration of AP significantly inhibits LH secretion. Opiate mechanisms are involved since naloxone pretreatment abolishes the effect. At least in part, the site of action of AP to inhibit LH release is in the median eminence, since catecholamine stimulation of LHRH release from median eminence explants can be reversed by AP. While in our hands, AP administration fails to alter growth hormone or thyroid stimulating hormone secretion, central injection of AP profoundly inhibits prolactin (PRL) secretion in conscious, male rats and AP infusion abolishes the "proestrous" PRL surge in females. No direct pituitary action of AP on PRL secretion can be demonstrated. A hypothalamic action of AP to stimulate dopamine (DA) turnover into the hypophysial portal vessels is indicated by our demonstration that injection of the dopamine receptor blocker, domperidone, either prior to or following central injection of AP reverses the PRL inhibitory action of the peptide and prior treatment with alpha-methyl-p-tyrosine results in elevated PRL levels (removal of DA inhibition) that cannot be reversed by AP injection. Thus potent interactions of AP with neuropeptide and neurotransmitter systems have been described, suggesting significant neuromodulatory actions of the peptide within the brain.

A 023 ROLE OF ATRIOPEPTIN IN CNS REGULATION OF THE CARDIOVASCULAR SYSTEM, C.B. Saper, Depts. of Pharmacological and Physiological Sciences and Neurology, University of Chicago, Chicago, IL 60637.

Atriopeptin (AP), the atrial natriuretic peptide, serves as a neuromodulator in the brain. Recent studies from our own laboratory and several others show that AP-like immunoreactive (-ir) nerve cells and axons are found in a characteristic distribution in the rat brain. Molecular studies of the immunoreactive peptide and its mRNA precursor indicate that the AP-ir material in the brain is authentic AP. In situ hybridization using a riboprobe for the AP gene shows AP gene transcription in hypothalamic cell groups that are AP-ir. AP-ir neurons are found in two main groups of structures: cell groups concerned with central cardiovascular control, and the limbic system. We have examined the anatomy and physiology of AP in the central cardiovascular regulatory system to determine the role that this peptide plays in these cell groups. The paraventricular nucleus of the hypothalamus (PVH) receives a major AP-ir projection that originates primarily from neurons adjacent to the anteroventral third ventricle. This latter area has been implicated in the control of blood pressure, volume and electrolyte composition. The projection includes the parvocellular parts of the PVH (involved in autonomic and anterior pituitary control) and its magnocellular parts (containing neurons that secrete oxytocin and vasopressin from their terminals in the posterior pituitary gland). Application of AP to vasopressin neurons in the magnocellular PVH was found to result in profound inhibition of firing, consistent with the role of AP systemically in opposing vasopressin effects. AP-ir neurons in the lateral hypothalamic area project both to the spinal cord, where they may innervate sympathetic preganglionic neurons, and to the nucleus of the solitary tract (NST). Preliminary experiments applying AP to NST neurons show that the peptide may differentially affect the processing of cardiovascular (such as carotid sinus nerve) as compared to other inputs to NST neurons. AP-ir neurons participate in a variety of important central autonomic control pathways, in which their peptide content may code for neuronal projections connections that are involved in cardiovascular regulation. AP in the brain appears to be involved in controlling the same functions that it regulates as a systemic hormone.

Experimental Pathophysiology

A 024 ATRIAL NATRIURETIC FACTOR IN CONGESTIVE HEART FAILURE. J. C. Burnett, Jr., B. S. Edwards, M. M. Redfield, W. S. Miller, R. S. Zimmerman, and D. M. Heublein. Mayo Medical School, Rochester, Minnesota

Congestive heart failure (CHF) is a syndrome characterized by chronic atrial stretch with increased circulating ANF in association with avid sodium retention and activation of the renin-angiotensin-aldosterone system. Despite the increase in atrial pressure and ANF in CHF, the increase in ANF may be inappropriately low for the level of increased atrial pressure. Thus, a relative deficiency in ANF may exist in chronic CHF with an attenuated stimulus-release relationship. In addition to enhanced synthesis and release of ANF from the atria in CHF, ventricular myocytes dedifferentiate to produce immunoreactive ANF in both animals and humans with CHF.

The physiologic significance of activation of the atrial peptide system in CHF remains controversial. Studies from our laboratory support the hypothesis that increased circulating ANF in acute CHF promotes sodium excretion and inhibits the RAAS. In chronic CHF, ANF may provide relative preservation of renal hemodynamic and excretory function opposing the action of vasoconstrictive-sodium retentive hormones activated in chronic CHF.

A therapeutic role for exogenous ANF in the treatment of CHF remains unclear. We have observed that low dose infusion of ANF in humans with CHF results in a selective renal action characterized by an increase in glomerular filtration rate and renal blood flow in association with inhibition of renin release. Thus, ANF may emerge as adjunct therapy in the treatment of congestive heart failure.

A 025 BIOSYNTHESIS SECRETION AND EFFECT OF ATRIAL NATRIURETIC POLYPEPTIDE (ANP) IN CONGESTIVE HEART FAILURE (CHF) IN MAN

Kazuwa Nakao, Akira Sugawara, Hiroshi Itoh, Yoshihiko Saito, Hiroshi Arai, Narito Morii, Takayuki Yamada, Masashi Mukoyama, Kiminori Hosoda, Gotaro Shirakami and Hiroo Imura, Second Division, Department of Medicine, Kyoto University School of Medicine, Kyoto 606, Japan.

The heart is an endocrine organ secreting ANP as well as a pump organ. Growing evidence indicates that the endocrine function, or ANP secretion from the heart is increased in the CHF, or the failing state of the pump function.

In order to elucidate the clinical implication of ANP in CHF, we have studied biosynthesis and secretion of ANP in human failing hearts with the aid of Northern blot hybridization technique and two radioimmunoassays (RIAs) for α-human ANP and for N terminal fragment of γ-human ANP, γ-human ANP [1-25], and also examined effects of ANP infusion in patients with congestive heart failure.

ANP messenger RNA (ANPmRNA) levels in right auricles from severe CHF (NYHA III, IV) were higher than those from mild CHF (NYHA I, II). Atrial ANP concentrations in NYHA III and IV (248.5 ± 69.9 μg/g) were higher than those in NYHA I and II (52.6 ± 14.9 μg/g). HP-GPC and RP-HPLC coupled with RIA showed that the predominant component of ANP is γ-human ANP (13K) in the mild CHF whereas α-human ANP (3K) and/or β-human ANP (6K) are prevailing in severe CHF. In addition, increased ANPmRNA and ANP levels were also demonstrated in ventricles of CHF obtained at autopsy.

γ-human ANP-derived peptides in normal human plasma consisted of two components, α-human ANP and 10K N-terminal fragment (N-peptide). In addition to these two components, γ-human ANP and β-human ANP were also detected as minor components in plasma from CHF. β-human ANP was converted into α-human ANP in human plasma in vitro and in vivo. Plasma ANP and N-peptide concentrations manifested concomitant and graded rises in accordance with the severity of CHF.

The ANP infusion significantly decreased pulmonary capillary wedge pressure from 23.8 ± 1.1 mmHg to 15.2 ± 3.1 mmHg and increased stroke volume index from 26.2 ± 4.2 ml/m^2 to 31.6 ± 4.1 ml/m^2 in CHF of NYHA III and IV. The ANP infusion increased urine volume, excretion of sodium and endogenous creatinine clearance and decreased the plasma aldosterone level. The ANP infusion also caused a significant reduction of the plasma N-peptide level in parallel with the improvement of left ventricular function.

Thus, biosynthesis and secretion of ANP are increased in human failing hearts and ANP infusion improves left ventricular function in CHF, suggesting that augmented biosynthesis and secretion of ANP in the failing heart are its own compensatory mechanisms of the heart.

A 026 BIOLOGICALLY ACTIVE ATRIAL NATRIURETIC PEPTIDES SELECTIVELY ACTIVATE Na/K/Cl COTRANSPORT IN VASCULAR SMOOTH MUSCLE CELLS, †Nancy E. Owen, †Martha E. O'Donnell, §Eugene N. Bush and §William Holleman, †Department of Biological Chemistry and Structure, The Chicago Medical School, North Chicago, Illinois and §Abbott Laboratories, Abbott Park, Illinois.

Atrial natriuretic peptides (ANPs) cause vasorelaxation, natriuresis, and diuresis. Although the precise mechanism of action for these biological activities is not known, it has been established that ANPs can bind to specific membrane receptors and can cause an increase in intracellular cyclic GMP (cGMP) levels. In previously published studies we have probed the mechansim of action of ANP and have shown that one consequence of ANP receptor-mediated increases in cGMP in vascular smooth muscle cells is stimulation of Na/K/Cl cotransport (1,2). Although others have suggested that ANPs may affect Na/H exchange and/or Na/K ATPase activity in various cells and tissues, the effect of ANPs on these other Na transport systems in VSMC is not known. Furthermore, the biological relevance of ANP-stimulation of Na/K/Cl cotransport in VSMC has not been established. The goal of the present study was to investigate whether ANPs selectively stimulate Na/K/Cl cotransport in VSMC and to determine whether effects on cotransport parallell biological activity. We tested the effect of six ANPs on Na/K/Cl cotransport, and one ANP on Na/H exchange and on Na/K ATPase activity. It was found that ANPs stimulated Na/K/Cl cotransport but had no effect on Na/H exchange or on Na/K ATPase activity in VSMC. Biological activity of the ANPs was assayed by measuring the potencies for producing vasorelaxation of aortic rings and for stimulating an increase in intracellular cGMP in VSMC. The rank orders observed for the two biological activities agreed with the rank order for stimulation of Na/K/Cl cotransport. It is concluded that ANPs selectively activate Na/K/Cl cotransport in VSMC and this activity may be related to the biological activity of these peptides.

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2. O'Donnell, M.E. and Owen, N.E. (1986) *J. Biol. Chem.* 261: 15461-15466.

A 027 VENTRICULAR ATRIOPEPTIN SYNTHESIS IN CHRONIC CARDIAC OVERLOAD, Paul T. Stockmann, Donald H. Will*, Roger C. Wiegand* and Philip Needleman. Department of Pharmacology, Washington University School of Medicine and *Monsanto Company, Biological Sciences Department, St. Louis, MO 63110.

Atriopeptin (AP) is released into the circulation in response to pressure or volume overload. Recent reports have demonstrated AP synthesis in nonatrial tissues including the cardiac ventricle. Hypertrophy produced by cardiac overload appears to induce AP synthesis in the ventricle. We examined three models of cardiac overload to define the relationship between hypertrophy and ventricular AP synthesis. Aortic banding (AB) and hypoxia (HYP) produced pressure overload of the left and right ventricle respectively. Chronic volume overload was produced by aortocaval fistula (AVF). Ventricular tissues were extracted by boiling in 1N acetic acid. Atriopeptin immunoreactivity (APir) of the extracts was determined by enzyme immunoassay. AP mRNA was quantitated by solution hybridization using a ^{32}P labelled mRNA probe. Aortic banding produced left ventricular (LV) hypertrophy and a significant increase in APir (7-fold over controls) and AP mRNA (5-fold) only in the LV. Hypoxia resulted in hypertrophy primarily of the right ventricle (RV) with a 9-fold increase in RV APir and a 160-fold increase in RV AP mRNA. Moderate hypertrophy and increased APir and AP mRNA was also present in the LV. Aortocaval fistula demonstrated marked biventricular hypertrophy with a 7-9 fold increase in ventricular APir and a 6-8 fold increase in ventricular AP mRNA. Purification of ventricular extracts by HPLC revealed primarily the high molecular weight prohormone in all three models.

MODEL	AB	HYP	AVF	CONTROL
LV				
APir (ng/mg protein)	22.6 ± 6.6	13.4 ± 1.4	27.9 ± 4.2	2.8 ± 0.2
AP mRNA	5.4 ± 1.7	8.0 ± 2.6	6.5 ± 0.9	1.0
RV				
APir (ng/mg protein)	3.6 ± 0.5	18.1 ± 4.1	15.2 ± 8.2	1.9 ± 0.2
AP mRNA	1.0 ± 0.2	163.3 ± 38.3	7.9 ±2.6	1.0

These data demonstrate that hypertrophy produced by pressure or volume overload stimulates atriopeptin synthesis by the ventricle. Ventricular myocyte stretch may represent the stimulus for AP synthesis in the ventricle. The mechanisms and magnitude of ventricular AP release remain to be elucidated.

Biological and Molecular Aspects of Atrial Factors

Cardiovascular II

A 028 PULMONARY ATRIAL PEPTIDE, Mark G. Currie, Catherine E. Lofton, David A. Baron, William F. Oehlenschlager and David Kurtz, Dept. of Cell and Molecular Pharmacology, Medical University of South Carolina, Charleston, S.C. 29425

We have found that rat lung extract possesses atrial peptide immunoreactivity. Analysis of the pulmonary extract by reverse phase HPLC showed two major peaks (Peak I corresponds to the active hormone and Peak II to the prohormone). Immunocytochemical localization of the atrial peptide indicated its presence in the pulmonary epithelial cells. Extracts of dissected portions of the respiratory tree (trachea and 1° bronchus) were found to possess immunoreactivity and the presence of atrial peptide mRNA was confirmed by dot-blot analysis. Investigation of tissue localization was pursued by obtaining an enriched population of airway epithelial cells by manual scraping of dog tracheal wall. Isolated canine tracheal epithelial cells were found to contain measurable levels of atrial peptide immunoreactivity. HPLC analysis of extracts from tracheal epithelial cells revealed a peak corresponding to the active hormone peak from canine atrial extract. Furthermore, we have observed that hamsters (1 year of age) in congestive heart failure have a 10-fold increase of pulmonary atrial peptide levels compared to age matched controls. These findings suggest that the pulmonary epithelial cells are a source of atrial peptide and that the production of pulmonary atrial peptide may have a role in the pathophysiology of pulmonary edema in such conditions as congestive heart failure. Future studies will involve establishing a physiological role for atrial peptide in pulmonary epithelial transport and investigating possible relaxant effects on bronchial smooth muscle in both normal and disease states.

Biosynthesis and Regulation

A 029 ISOLATION AND CHARACTERIZATION OF A NOVEL ATRIAL PEPTIDE WITH HYPOTENSIVE ACTIVITY, T.G. Flynn, Anoop Brar, David Hyndman, Christine Lyons, Linda Tremblay and D.B. Jennings*, Departments of Biochemistry and Physiology*, Queen's University, Kingston, Ontario, Canada K7L 3N6.

High performance liquid chromatography (HPLC) of acidic extracts of rat atria results in the separation of several peptides including pro atrial natriuretic factor (pro-ANF) and several truncated forms of pro-ANF. We have isolated, purified and sequenced a novel atrial peptide which co-migrates with a truncated form of pro-ANF. This peptide has a molecular weight of about 4500 and a unique amino acid sequence. When injected into rats with a positive sodium balance and maintained with a constant saline infusion the peptide caused a profound fall in blood pressure and a decrease in the heart rate. Injection of the peptide in doses of 2-3 µg had no effect on sodium or water excretion whereas comparable doses of ANF cause significant increases in urine and sodium excretion. The discovery of this peptide, which we have named atrial hypotensive peptide (AHP), demonstrates that the heart contains peptides other than ANF and that these peptides may play a role in the control of blood pressure. AHP may work independently or in concert with ANF.

Biological and Molecular Aspects of Atrial Factors

Biosynthesis and Regulation

A 100 TISSUE-SPECIFIC EXPRESSION OF THE HUMAN ANF GENE, M.C. LaPointe, J.P. Wu, D.G. Gardner. Dept. of Medicine and Metabolic Research Unit, Univ. of Calif., San Francisco San Francisco Ca. 94143 5'-flanking sequences (5'fs) from the human atrial natriuretic factor (ANF) gene were subcloned into a promoterless vector containing the chloramphenicol acetyltransferase (CAT) reporter sequence. These hybrid constructions were transfected into primary cultures of neonatal rat atrial cardiocytes, ventricular cardiocytes or non-myocardial cells and assayed for CAT activity 62-68 hrs later. In atrial cells, hybrid genes containing from 2500 to 409 base pairs (bp) of 5'fs gave the highest level of CAT expression. Deletion from -409 to -332 resulted in a significant decrease in expression of the reporter gene. A similar pattern of expression was seen in ventricular cardiocytes. Expression of the hANF-CAT constructs was tissue-specific. No expression was seen in primary cultures of non-myocardial cells from neonatal rat hearts, or in GC cells, a rat pituitary tumor cell line. To determine whether this tissue-specificity might arise from interaction of these ANF sequences with specific nuclear proteins, DNA fragments from the 5'fs were tested for their ability to bind to protein in extract of myocardial or non-myocardial cells using a "gel shift" assay. The results indicated that a 192bp PvuII fragment from the 5'fs of the hANF gene (-423 to -240) bound to protein factors in a tissue-specific fashion. No binding was identified when extracts from non-myocardial cells were employed. Competition experiments indicated that binding to the 192bp fragment was sequence specific. 0.4ng of unlabeled PvuII fragment competed with radiolabeled fragments for binding to the protein extract. Neither a 229bp fragment from hANF intron B, nor a 200bp fragment from the hGH cDNA displaced the labeled PvuII fragment from the protein. These findings demonstrate that the DNA sequence between -332 and -409 in the hANF gene harbors a tissue-specific element, and that a portion of this element's function may depend on its ability to associate with a cardiac specific nuclear protein.

A 101 VENTRICULAR ATRIAL NATRIURETIC FACTOR GENE EXPRESSION: A MARKER FOR CARDIAC HYPERTROPHY, Mona Nemer, Jean-Pierre Lavigne and Jacques Drouin, Clinical Research Institute of Montreal, Montreal, Quebec, CANADA H2W 1R7

In the normal adult heart, ANF is mostly synthesized in atria although ventricles contain a low level of ANF mRNA and peptide. Since cardiac hypertrophy is associated with several subcellular and biochemical changes in the ventricles including appearances of granules, we examined the expression of the ANF gene in genetic, experimental and pathological cardiac hypertrophy. In all animal models and human subjects that we examined, cardiac hypertrophy was accompanied by a marked induction of ANF gene expression in both ventricles. Bio 14.6 hamsters develop inheritable cardiomyopathy which results in cardiac hypertrophy and eventually congestive heart failure (CHF). In these animals, progression of hypertrophy correlates with increases in ventricular ANF mRNA levels: increases of about 10-fold and 30 to 50-fold are observed in both ventricles around ages 100 and 200 days, respectively. In SHR rats, development of high blood pressure is associated with increases of ventricular mass and ANF mRNA concentrations; in 11 week old animals, right and left ventricular ANF mRNA levels are already 3-fold higher in SHR than in control WKY rats. Glucocorticoid administration which produces mild cardiac hypertrophy also induces a 4-fold increase in left ventricular ANF mRNA accumulation in rats. Similar induction of ventricular ANF gene expression is observed in human subjects with cardiac hypertrophy resulting from glucocorticoid Rx, hypertension or CHF. Thus, ventricular ANF gene expression is a very sensitive parameter for the analysis of biochemical events which trigger cardiac hypertrophy.

A 102 **PRODUCTION OF ANTIBODIES TO THE ANP C-RECEPTOR USING VACCINIA VIRUS RECOMBINANTS**
J. Gordon Porter, Yu Wang, Karen Schwartz, Ann Arfsten, Kaye Spratt, Beverly Dale, Robert M. Scarborough, and John A. Lewicki, California Biotechnology, Inc.
2450 Bayshore Parkway, Mt. View, CA 94043

A recombinant vaccinia virus containing cDNA sequences corresponding to the bovine ANP C-receptor has been isolated and receptor expression characterized as previously described (Schenk et al., 2nd BAAP). The ability of this vector to infect a variety of hosts has been exploited by inoculating rabbits with living virus. New Zealand white rabbits were immunized intradermally with scarification with 10^8 PFU of recombinant virus and bled 2 weeks later. Serum titers were tested by immunoprecipitation. The serum samples immunoprecipitate a 65 kd protein (ANP C-receptor) specifically from recombinant vaccinia infected L-cell lysates. The antisera also immunoprecipitates ^{125}I-ANP crosslinked expressed receptor from the same infected cells and crosslinked native receptor from bovine aortic smooth muscle (BASM) cells. In addition, this antisera to the ANP C-receptor inhibits the binding of ^{125}I-ANP to BASM cells and to expressed receptor solubilized with detergent. This reagent will prove useful in dissecting the roles of different ANP receptor populations. This report demonstrates the utility of the vaccinia vector system in generating antibodies to a cloned heterologous protein.

Hormone Processing and Peptide Release

A 103 IMMUNOELECTRON MICROSCOPICAL LOCALIZATION OF ANF IN FETAL, NEONATAL, AND ADULT ATRIOCYTES WITH REGARD TO THE DYNAMIC OF SECRETORY PATHWAY, * Jacques Gilloteaux, ** Roberte Menu, + Lothar Jennes, and ** Jean-Jacques Vanderhaeghen, * Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272, ** Free University of Brussels, Brussels, Belgium, and + Wright State University, Dayton, OH 45435.
An antibody raised against the N-terminus fragment of ahANF (atriopeptin III) was able to cross-react and to label specifically the content of atrial granules in fetal, neonatal, and adult golden Syrian hamsters by using immunogold electron microscopic technique adapted from DeMey (1983). The content of the atrial granules was labeled from its site of formation to exocytosis into the subendocardial spaces. These were observed for all age groups; then labeling was found at the level of vesicles or non-specialized structures of the endocardial endothelial cells before ANF-IR product was demonstrated to be released in the atrial chamber by exocytotic vacuole. In the cases of neonatal and adult groups, where atrial trabeculae myofibers were invaded by blood vessels, ANF-IR compound also appeared in the endothelial cells, at their microvillar surface, and released into the blood stream by exocytotic events. Immunogold labeling was significantly more abundant in the atrial granules of the oldest atria. This report suggests that the endothelial cells could play an active role either in the activation and/or in the release of the ANF into the circulation. Supported by Ohio Research Challenge fund to NEOUCOM and FNRS of Belgium, and Am. Heart Assoc. Ohio.

A 104 ASSOCIATION OF ORNITHINE DECARBOXYLASE WITH RAT ATRIAL GRANULES CONTAINING ATRIAL NATRIURETIC PEPTIDE, Mari K. Haddox, Alton L. Steiner, Charles Skiera and Ulka R. Tipnis, University of Texas Medical School, Houston, Texas, 77225.
Ornithine decarboxylase (ODC) is the initial rate-limiting enzyme in the biosynthesis of polyamines. A polyclonal antibody elicited against ODC was used to analyze the intracellular distribution of the enzyme protein within atrial myocytes at the ultrastructural level through the use of a post-embedding immunogold technique. In sections prepared from saline-injected animals, gold particles were detected in association with all the subcellular structures. The density of gold particles associated with atrial granules was 7 to 30-fold higher than that detected in association with the other subcellular compartments. Six hours after the administration of isoproterenol the atrial ODC activity increased 20-fold and the density of the immunolabeling of the atrial myocytes increased 5-fold. Statistically significant increases in the density of subcellular labeling occurred in association with the atrial granules and the nucleus. There was an increase in both the number of atrial granules which were labeled by the anti-ODC immune reaction and in the density of the gold particles over individual granules. Based on morphometric calculations, sixty per cent of the immuno-detectable ODC protein in the atrial myocytes was associated with the atrial granules. All of the granules in the atrial myocytes contained atrial natriuretic peptide immunoreactive material. These results suggest consideration of a potential role of ODC activity and/or polyamines in the synthesis, processing or secretion of atrial natriuretic peptide.

A 105 ATRIAL GRANULES CONTAIN A PROCESSING ENZYME OF PRO-ATRIAL NATRIURETIC FACTOR, Robert B. Harris and Donna M. Wypij, Virginia Commonwealth University, Richmond, VA 23298-0614.
At least three enzymes have been identified in atrial tissue homogenates that are capable of processing pro-atrial natriuretic factor (pro-ANF) to active atrial peptides. We have isolated and characterized a serine-proteinase associated with atrial granules that preferentially hydrolyzes the Arg-Ser bond in the substrates, Gly-Pro-Arg-Ser-Leu-Arg, Bz-Gly-Pro-Arg-Ser-Leu-Arg and Bz-Gly-Pro-Arg-Ser-Leu-Arg-Arg-2-naphthylamide (NA), the Arg-2-NA bond in the substrate Bz-Gly-Pro-Arg-2-NA, and the Arg^{98}-Ser^{99} bond in a 31-residue substrate, Gly^{96}-Tyr^{126}. This latter peptide contains the putative processing site in pro-ANF and the sequence for the bioactive peptides. Our results indicate that the Ser^{99}-Tyr^{126} natriuretic peptide is the predominate hydrolytic product but after prolonged incubation or at high enzyme concentrations, the Ser^{103}-Tyr^{126} natriuretic peptide may also be formed. The Ser^{103}-Arg^{125} natriuretic peptide was only a very minor product. The minimum processing site sequence is Gly-Pro-Arg-Ser-Leu-Arg-Arg but the doublet of basic amino acids is not the primary processing site in pro-ANF. Our findings are consistent with the idea that the pro-protein and the processing enzymes are packaged into the secretory granule and in response to the proper stimulus, the pro-protein is processed to the active peptides.

A 106 RELEASE OF ATRIAL NATRIURETIC FACTOR IS MODULATED BY OPIOIDS, Angelika M.Vollmar and Rüdiger Schulz,University of Munich,8000 Munich 22,FRG

The release of ANP into circulation is stimulated by a number of drugs which are directly or indirectly involved in the regulation of water and electrolyte balance. Since opioids, dependent on their receptor specificity, are known to also control water balance in animals and man, we studied the effect of multiple opioid receptor agonists on plasma immunoreactive atrial natriuretic peptide (IR-ANP) in conscious non-hydrated rats. Mu-opioid receptor agonists which decrease urination, increased plasma IR-ANP. In contrast, kappa-receptor agonists, provoking a diuresis, did not affect plasma levels of ANP. The mu-agonist fentanyl (0.05 mg/kg) caused a 10 fold increase in IR-ANP plasma concentration. Maximal levels were reached within 5-10 min. The selective kappa receptor ligands U 50.488-H and ethylketocyclazocine failed to elicit an IR-ANP response. Pretreatment with the narcotic antagonist naltrexone (0.05 mg/kg, 20 min) completely blocked the fentanyl effect upon plasma IR-ANP, proving the opioid receptor specificity of the fentanyl activity. The fentanyl-induced increase of ANP was not abolished by the quaternary antagonist N-methylnaltrexone (1 mg/kg, 20 min),suggesting that opiates bring about their action on ANP via a central mechanism.

A 107 METABOLIC FATE OF ATRIAL NATRIURETIC POLYPEPTIDE. L. Wennogle, R. Ghai, C. McMartin, J. Sonnenberg, R. Webb, and M. Zimmerman. Research Department, CIBA-GEIGY Corp., Summit, New Jersey, 07901. The metabolic fate of rat atrial natriuretic polypeptide [rANP (1-28)] was studied after systemic administration. Bolus injection of different radiolabeled peptides was followed by rapid metabolism. Disappearance of ^{3}H-ANP was assessed by radio-HPLC and found to follow biphasic pharmacokinetics (t1/2 alpha = 0.19 min, t1/2 beta = 1.0 min; V_1 = 9.6 ml, V_{ss} = 16.5 ml) with a clearance of approximately 15 ml/min. Proteolytic mechanisms were found to have a high capacity for clearance, as doses of peptide sufficient to occupy receptors are none-the-less rapidly converted to circulating fragments. Of several proteases studied and characterized, the predominant activity found in rat kidney was capable of cleaving specifically between the Cys7-Phe8 bond. This enzyme was purified and identified as protease 24.11, also known as enkephalinase. The selectivity and tissue distribution of the enzyme will be discussed.

A 108 A CARDIAC HYBRIDOMA CELL LINE PRODUCING ATRIAL NATRIURETIC POLYPEPTIDE (ANP), Xia Zhen-qin, Xie Xuan-Zhu, Tang Jian, Laboratory of Cardio-Pulmonary Endocrinology, Beijing Medical University, Beijing, China.

The atrial cardiocytes can produce and secrete ANP but cannot be cultured permanently and proliferated repeatedly in vitro. In the present study, we have established a cardiac hybridoma cell line, CP8401, from newborn BALB/c mouse cardiac cells fused with NS1 myeloma cells which can produce and secrete i-ANP continuously. The CP8401 cells proliferate rapidly in vitro as well as in vivo in the abdominal cavity of BALB/c mice. The average number of the chromosomes of the CP8401 cells is 89 per cell. Using specific immunocytochemical technique for ANP, the granules containing immunoreactive ANP were clearly observed in the CP8401 cells, but not in NS1 cells. When the anti-ANP antiserum was preabsorbed with rANP (15ug), the positive staining granules in the CP8401 cells were abolished. Using the method of sensitive and specific ANP RIA, it was found that the concentration of i-ANP in the CP8401 cells and in the supernate of the culture were about 9 pg/10 ^{7} and 380 pg/10 ^{7} cells respectively. The supernate of the cells was collected and separated on C18 column and radioimmunoassayed for ANP. One main peak of i-ANP was collected and further purified by reverse-phase HPLC. Two main peaks of i-ANP were eluted similarly to the artificial synthesized atriopeptin I and rat ANP. When the sample purified by HPLC was injected into the rat, it was observed that the urine volume of the rat increased 5.5 fold at 60 minutes after injection. These results indicate that the CP8401 cell line can produce and secrete ANP and show that it is possible to establish endocrine hybridoma cell lines for the production of ANP and for study of ANP release and its regulation.

Receptors

A 109 PURIFICATION AND CHARACTERIZATION OF THE ATRIAL NATRIURETIC FACTOR RECEPTOR FROM BOVINE ADRENAL CORTEX, Sylvain Meloche, Normand McNicoll, Huy Ong and André De Léan, Clinical Research Institute of Montreal, Montreal, Canada H2W 1R7.

Atrial natriuretic factor (ANF) potently inhibits aldosterone secretion by interacting with specific high affinity receptors in adrenal zona glomerulosa membranes. Quantitative analysis of ANF binding data has suggested the presence of two affinity states of the ANF receptor, a high affinity state (pK 11.2) which can be promoted by the diuretic amiloride and a low affinity state (pK 9.8). However, results from affinity labeling studies are consistent with the notion of a single receptor protein of Mr 130,000 (Meloche et al., J. Biol. Chem. 262:10252, 1987). In order to understand the structure and functional properties of the ANF receptor, we have developed a procedure for the purification of the receptor from bovine adrenal zona glomerulosa. The ANF receptor was first solubilized with Triton X-100 and adsorbed on a QMA anion exchange column. Following elution at high ionic strenght, the receptor was subjected to affinity chromatography on an ANF-agarose matrix. The receptor was eluted by acidic buffer, concentrated, and further purified by steric exclusion high performance liquid chromatography. SDS-polyacrylamide gel electrophoresis and silver staining of the purified receptor under reducing conditions revealed the presence of a single protein of Mr 130,000. The purified receptor showed the same affinity for ANF analogs as the native receptor of crude membranes and was still sensitive to modulation by amiloride.

A 110 A TRUNCATED, LINEAR ATRIAL PEPTIDE (AP) ANALOG THAT DISCRIMINATES BETWEEN TWO CLASSES OF RECEPTORS IN RABBIT LUNG, Gillian M. Olins, Dennis R. Patton, Philippe R. Bovy and Pramod P. Mehta, Searle Research & Development, Chesterfield, MO 63198

Competitive binding studies using rabbit lung membranes showed that AP(103-126) and a truncated linear AP analog, des($Cys^{105,121}$) AP(104-126) (referred to as analog I) displaced bound ^{125}I-AP(103-126) from specific AP binding sites 100% (K_i of 0.28 nM) and 73% (K_i of 0.30 nM), respectively. Radioiodinated AP(103-126) and analog I were chemically cross-linked to binding sites on rabbit lung membranes, and the labelled membrane proteins were analyzed by SDS-PAGE in the presence and absence of 2-mercaptoethanol. ^{125}I-analog I specifically labelled a 65 KD protein, and a 135 KD protein which, under reducing conditions, dissociated into 65 KD subunits. In contrast, ^{125}I-AP(103-126) labelled specifically a nonreducible 135 KD protein, in addition to the 65KD species and the reducible 135 KD species. AP(103-126) stimulated rabbit lung particulate guanylate cyclase activity, whereas analog I had no effect on cGMP production, and did not antagonize the effect of AP(103-126). Thus, analog I appears to be a selective ligand which binds to approximately 73% of the total AP binding sites, and does not activate guanylate cyclase. Binding to the cyclase-linked AP receptor correlates with the specific labelling by ^{125}I-AP(103-126) of the nonreducible 135 KD membrane protein.

A 111 LINEAR FRAGMENTS OF ANP'S BIND TO C-ANP RECEPTORS WITH HIGH AFFINITY R.M. Scarborough, A. Arfsten, L.L. Kang, K. Schwartz, G.A. McEnroe, J.G. Porter, M. Suzuki, T. Maack and J.A. Lewicki, California Biotechnology, Inc., Mt. View, CA 94043 and the Dept. of Physiol., Cornell University Medical College, NY, NY 10021.

C-ANP receptors constitute the overwhelming population of binding sites for the ANP's in cultured vascular cells and the isolated perfused kidney (IK). We have recently shown that [des(18-22)]-rANP (4-23)-NH_2 specifically binds with high affinity only to C-ANP receptors and not to ANP receptors coupled to direct biological effects (Maack et. al. Science, in press). We have also proposed that the major function of C-ANP receptors is to serve as specific storage-clearance binding sites for endogenous ANPs. Since the structural requirements for interaction of ANPs with C-ANP receptors appears to be quite unique, we have attempted to define the pharmacophore contained within the ANPs responsible for interaction with C-ANP receptors. In the present study, analogs of [des(18-22)]-rANP(4-23)-NH_2 were prepared that include removal of amino-terminal R-S-S residues, replacement of Cys residues with Ala and truncations of these linear ANP fragments. It was determined the linear analogs retain high affinity for C-ANP receptors in cultured vascular cells (K_i 2-15nM) and in IK. Thus, the smallest fragment with native ANP amino acid sequence which retains high affinity for C-ANP receptors is the octapeptide, rANP (8-15)-NH_2. These findings underscore the unique specificity of C-ANP receptors and, furthermore, suggest that any full-length ANP analogs which retain all or a portion of the residues (8-15) will bind to C-ANP receptors, leading to their rapid elimination from the circulation as has been shown for ANP (1-28).

A 112 PRIMARY STRUCTURE OF THE ANF RECEPTOR, A MEMBRANE-BOUND, 140-KDA GLYCOPROTEIN THAT STIMULATES GUANYLATE CYCLASE, Valerie M. Watt, T. Geoffrey Flynn and Cecil C. Yip, Univ. of Toronto, Toronto and Queen's Univ., Kingston, Ontario, Canada. Atrial natriuretic factor (ANF) binds specifically to two size classes of receptors: the higher M_r receptors (M_r 110,000 to 180,000) mediate ANF action by stimulating particulate guanylate cyclase. To gain insight into the mechanism by which this ANF receptor mediates the responses to ANF, we have isolated and determined the nucleotide sequence of cDNA clones encoding the high M_r ANF receptor in rat kidney. DNA encoding the ANF receptor was isolated first from a λgt11 library using serum raised against the high M_r ANF receptor and subsequently by hybridization to the serum positive clones. We have established that the initial isolates encode the ANF receptor; antibodies selected from the immune serum by the fusion protein produced by these phage detect a protein band of 140 kDa, precipitate the photolabelled ANF receptor, inhibit the specific binding of ANF, and stimulate guanylate cyclase activity. The DNA sequence encoding the ANF receptor predicts a high M_r receptor of 109,461 M_r in agreement with that estimated for the photoaffinity labelled receptor after deglycosylation. The predicted amino acid sequence for the ANF receptor is largely hydrophilic, with a single N-terminal hydrophobic domain which may act as a signal as well as a transmembrane domain. The absence of a discernable GTP-binding site in our predicted sequence would suggest that the ANF receptor protein of high M_r is distinct from that having guanylate cyclase activity. The availability of this cDNA encoding the ANF receptor provides a valuable tool for determining how the binding of ANF to its receptor can lead to activation of guanylate cyclase.

Endocrine Interactions and Intracellular Mediators

A 113 **ANF Dependent cGMP Responses in Aortic Endothelial and Smooth Muscle Cells Occur Through Different Receptor Subtype Populations**, G.P. Budzik, S.L. Firestone, T.W. Rockway and W.H. Holleman, Abbott Laboratories, Division of Cardiovascular Research, Abbott Park, IL 60064. Aortic endothelial cells express receptors for atrial natriuretic factor, ANF[1-28], and produce cGMP following stimulation with ANF peptides. We have assessed the potency of ANF analogs to provoke a cGMP response in bovine transformed aortic endothelial cells (BTAEC). Total cGMP was determined by RIA in cultures exposed to ANF or an analog for 2 hrs at 37°C. Maximum stimulation by atriopeptin-III, ANF[5-28], was set at 100% for standardization. Results reported as pD_2 values represent the -log of the concentration yielding a 50% cGMP response. The pD_2s for ANF analogs were: ANF[1-28] 8.30; ANF[5-28] 8.20; ANF[5-25] 5.35; Tyr-8 ANF[5-27] 6.53; ANF[7-23], Lys-11 ANF[7-23], and Thr-18 ANF[7-23], all <5.00. These results are notably different than we reported (BBRC 144:422-431 (1987)) for rabbit aorta vascular smooth muscle cells (VSMC). In VSMC, cGMP response curves with ANFs [1-28], [5-28] and [5-25] are shifted 10-100 fold to higher concentration, while Tyr-8 ANF[5-27] lacks activity and [7-23] analogs are equipotent with ANF[5-28]. Crosslinking of ^{125}I-ANF to BTAEC identifies 3 ANF receptor forms: a 65KD monomer, a 150KD multimer reducible with 2-ME to 65KD, and a non-reducible 150KD protein. Inhibition of crosslinking by co-incubation with selective analogs suggests that the non-reducible 150KD component is linked to cGMP production. VSMC contain only the 65KD and reducible 150KD components, although cGMP generation can be stimulated. These different ANF receptor subtypes may thus provide a basis for the differential cGMP stimulations by ANF analogs in BTAEC and VSMC.

A 114 ATRIAL NATRIURETIC PEPTIDE (ANP) CAUSES CALCIUM-DEPENDENT ELEVATION OF CYCLIC GMP LEVELS IN RAT BRAIN HIPPOCAMPAL SLICES. D.D. Kim, R.J. Maslowski, X. Wang and R.R. Fiscus. Dept. of Physiology, Loyola Univ. Medical Center, Maywood, IL 60153. Previous studies have shown that ANP causes Ca^{2+}-independent activation of particulate guanylate cyclase in homogenates of rat adrenal gland, aorta and kidney, but not whole brain. Yet, we still observed that atriopeptin II (AP-II) causes elevation of cGMP in rat hypothalamus, brain stem, and hippocampus (HPC). Also, we found that AP-II (100nM) increases cGMP levels (4-12 fold) in rat C6-2B glioma and PC12 pheochromocytoma cells. In addition, cGMP responses to AP-II in C6-2B cells were attenuated by chlorpromazine (CPZ) but not methylene blue (MB). Since these cGMP responses may be unique to transformed cells, we investigated effects of MB and CPZ in normal brain tissue. Following decapitation, brains were rapidly removed, placed in ice-cold KRB aerated with 95:5 and rapidly dissected at 0-4°C. Slices of HPC were selected as a model because they demonstrate the greatest and most consistent responses to AP-II. Time course and dose-response relationships of cGMP responses in HPC to AP-II, with or without IBMX, were similar to those in C6-2B and PC12 cells. After equilibration in KRB with 2.5 μM Ca^{2+} at 37°C for 1 hr, AP-II (100nM, 12 min) elevated cGMP by 3.5-6.0 fold. Preincubation with IBMX (250 μM, 10 min) enhanced responses by an additional 3-4 fold. In Ca^{+2} free KRB, responses to AP-II were abolished. However, preincubation with MB (10μM, 20 min) or CPZ (10 μM, 20 min), with or without IBMX preincubation, did not block the effects of AP-II. We conclude that the Ca^{+2}-dependent cGMP responses of AP-II is unique to neural tissues of the central nervous system. (Support: Grant 86 962 from the American Heart Association.)

A 115 ATRIAL NATRIURETIC FACTOR AND RAT ESTROUS CYCLE, Neil W. Hoffman, Allan A. MacPhee, and Arnold A. Gerall, Alton Ochsner Medical Foundation, New Orleans, LA 70121 and Tulane University, New Orleans, LA 70118.

Sodium and water retention vary accross the rat estrous cycle. In this study, cyclic comparisons were made among peripheral and central atrial natriuretic factor (ANF) concentrations. Sprague Dawley female rats, approximately 180 days of age maintained on a 14:10 hr light (onset = 500 hr): dark illumination cycle, were staged on the basis of vaginal cytology. Females (n = 7-13) were sacrificed by decapitation during early proestrus (900-1100 hr), late proestrus 1700-1900 hr), estrus (900-1100 hr), early metestrus (900-1100 hr), or late metestrus (1700-1900 hr). Plasma was obtained from trunk blood, and atria and brains were quickly removed and frozen in dry ice. Eight brain regions were dissected from frozen coronal sections (300 um) with a metal cannula (i.d. = 1 mm). Plasma and supernatants of atrial and brain regional homogenates were applied to Sep-Pak C18 cartridges, and extracted ANF concentrations were determined by radioimmunoassay. Plasma ANF concentration was significantly increased during early metestrus ($p < .01$), at which time atrial ANF was decreased relative to early proestrous ($p < .05$) and late proestrous levels ($p < .005$). Centrally, ANF was reduced during estrus in hypothalamic tissue surrounding the third ventricle ($p < .005$) and after proestrus in the dorsal raphe ($p < .05$). These findings suggest a relationship between female reproductive hormones and both peripheral and central ANF.

A 116 SECRETION AND BIOSYNTHESIS OF ATRIAL NATRIURETIC FACTOR BY CULTURED CHROMAFFIN CELLS, Tien-Tue Nguyen, Normand McNicoll, Huy Ong and André De Léan, Clinical Research Institute of Montreal, Montreal, Canada H2W 1R7.

Atrial natriuretic factor (ANF) is a peptide hormone secreted from mammalian atrial cardiocytes in response to increased right atrial pressure. Immunohistochemical studies have revealed the presence of ANF-like activity in the central nervous system, salivary gland, kidney, anterior pituitary and the adrenal medulla. We have also reported the existence of an ANF-like factor in acid extracts of bovine adrenal medulla (Life Sci. 36: 2375, 1985). We have further isolated and purified this peptidic factor and its precursor specifically localized in the bovine chromaffin granules (Biochem. Biophys. Res. Commun., in press, 1987). Amino acid analysis and sequence determination revealed a structure identical to bovine ANF(99-126) and its prohormone ANF(1-126) as previously purified from bovine atrial appendages (Life Sci. 38; 1309, 1986). We now report on the secretion and biosynthesis of ANF by cultured chromaffin cells. Exposure of chromaffin cells for 15 minutes to cholinergic nicotinic agonists or to depolarizing concentration of KCl induced an increase in the secretion of both the matured and the precursor form. Treatment of chromaffin cells for 3 days with phorbol ester increased the intracellular content of both forms of ANF activity. While forskolin did not affect the intracellular content, the combination of phorbol ester and forskolin produced a synergistic effect on the proANF activity. The results indicate a) that cultured chromaffin cells are a useful model for investigating the biosynthesis, maturation and secretion of ANF and b) the potential role of ANF as a neuropeptide which might exert a paracrine role in the adrenal gland.

A 117 PHOSPHORYLATION *IN SITU* OF ATRIAL NATRIURETIC PEPTIDE PROHORMONE AT THE CYCLIC AMP-DEPENDENT SITE, Judith Rittenhouse, Lorraine Moberly, Hazera Ahmed, and Frank Marcus. Department of Biological Chemistry and Structure, University of Health Sciences/The Chicago Medical School, North Chicago, IL 60064.

Previously we have shown that atrial natriuretic peptides (ANP) are excellent substrates for cAMP-dependent protein kinase, with phosphorylation occurring at Serine104 (Rittenhouse et al., 1986, J. Biol. Chem. 261:7607-7610). In continuing work, ANP prohormone has been found to be phosphorylated *in situ* after purification from rat atria incubated in the presence of ^{32}P-orthophosphate. The *in situ*-labeled prohormone was cleaved by thrombin into two fragments. The ^{32}P label was found to be associated with the COOH-terminal fragment that corresponds to processed ANP (Ser99-Tyr126), while the large NH_2-terminal fragment was unlabeled. Upon subcleavage of the (S-pyridylethylated) labeled fragment by chymotrypsin, reversed phase HPLC of the resulting peptides showed most of the label to have the same retention time as Arg-Arg-Ser-[PSer]-[Pyridylethyl-Cys]-Phe. The latter peptide was generated from similarly cleaved ProANP and synthetic ANP (Ser99-Tyr126) that had each been phosphorylated *in vitro* by cAMP-dependent protein kinase.

Our finding of phosphorylation of intracellular proANP at the cAMP-dependent site by adult atrial tissue contrasts with the recent report by Bloch et al., 1987, J. Biol. Chem. 262:9956-9961, who did not detect phosphorylation of the ANP portion of prohormone secreted by cultured neonatal rat atrial and ventricular cardiocytes. However, extensive phosphorylation was found at another site located in the amino terminal (non-ANP) portion of the prohormone. Thus, there appear to be two distinct sites in proANP that are differentially phosphorylated according to the source of material. (Supported by Grants from the American Heart Association with funds contributed in part by the Chicago Heart Association to JR and NIH DK 21167 to FM).

Renal Response and Mechanisms

A 118 EVIDENCE FOR ANF CONTROLLING SODIUM EXCRETION IN THE CONSCIOUS MONKEY. B.A. Benjamin, T. V. Peterson, C.H. Metzler, N.L. Hurst, and M.A. Maher. Texas A&M University, College of Medicine, College Station TX 77843 and University of California, San Francisco, CA 94143.

We have recently shown that atrial appendectomy (ATX) in the conscious monkey significantly attenuates the increase in ANF and sodium excretion after volume expansion (VE). In intact, control monkeys ANF increased with VE from 48±7 to 108±34 pg/ml and absolute and fractional sodium excretion increased from 17.9±2.6 to 74.9±12.0 uEq/min and from 0.67±0.10 to 2.43±0.28 %, respectively. In contrast, in ATX animals ANF failed to increase with VE (34±8 vs. 38±9 pg/ml) and absolute and fractional sodium excretion only increased from 19.3±6 to 37.8±5 uEq/min and 0.79±0.08 to 1.43±0.17 %, respectively. We have also shown in one monkey that ATX attenuates postprandial increases in sodium excretion. To better determine the role of ANF in these studies we repeated the VE in an ATX animal in which a replacement infusion of ANF (10 ng/kg/min) was administered when the VE was started. Urine flow and sodium excretion increased to levels normally attained by VE in control animals. ANF antiserum was then given during the peak renal response (while the ANF infusion continued) and urine flow and sodium excretion returned to control (pre-VE) levels. ANF antiserum administered to a control animal at the start of VE led to attenuated increases in urine flow and sodium excretion that were similar to the responses of the ATX animals. These findings, taken together, demonstrate that ANF plays a role in controlling sodium excretion in the conscious monkey. (Supported by NIH Grants HL31987, HL01383, HL35879 and Texas Heart Grant 85G-027).

A 119 ATRIOPEPTIN PRODUCES VASODILATION IN THE PERFUSED GILL OF THE MARINE TELEOST, *OPSANUS BETA*, David H. Evans & John A. Payne, Department of Zoology, University of Florida, Gainesville, FL 32611.

The comparative physiology of atriopeptin is relatively unstudied, especially in the lower vertebrates. Fishes face substantial, chronic, ionic and osmotic gradients across the relatively permeable gill epithelium, so one might expect that atrial peptides may be involved in fish osmoregulation. Since hemodynamics may control both the passive and active pathways for water and solute transport across the gill epithelium, it is of interest to examine the sensitivity of these vessels to atriopeptin. In addition, these vessels are the evolutionary precursors of the mammalian aorta, carotid, and coronary vessels, so study of their sensitivity has some relevance to questions about the origin and evolution of the atrial control of blood volume in the vertebrates. Intact gills were perfused with Ringer's containing specific concentrations of synthetic rat atriopeptin (101-126) after preconstriction with carbachol. Gill resistance was monitored via a pressure transducer in the afferent perfusion line. Concentrations above 10^{-10} M produced vasodilation, and the EC_{50} was 4 X 10^{-9}, in the range of that described for the vasodilatory action of atriopeptin on mammalian vessels. Acclimation of experimental fish to ca. 5% sea water, which produces chronic water loading, did not alter the concentration:response curve. These data indicate that the marine fish gill has receptors which recognize the $AP_{101-126}$, and which may control the pattern of blood flow through the gill vasculature. Supported by NSF PCM83-02621.

A 120 EFFECTS OF ACUTE INFUSION OF A C-ANP RECEPTOR SPECIFIC COMPOUND IN CONSCIOUS DOG L.C. Gregory, R.M. Scarborough, C.H. Metzler, G.A. McEnroe, T. Maack, and J.A. Lewicki, California Biotechnology, Inc., Mt. View, CA 94043 and the Dept. of Physiol., Univ. of Calif., S.F., CA 94143 and Cornell University Medical College, NY, NY 10021.

Specific ligands of C-ANP receptors increase plasma levels of endogenous ANP by decreasing the metabolic clearance rate of the hormone leading to natriuretic and hypotensive effects in intact anesthetized rats. In order to assess the cardiovascular and renal effects in a conscious animal, [des(18-22)]rANP(4-23)NH_2 (0.1-3.0 µg/kg/min., i.v.) was administered to conscious dogs. Renal parameters were tabulated as cumulative responses.

	Saline	0.1 µg/kg/min	0.3 µg/kg/min	1.0 µg/kg/min	3.0 µg/kg/min
Cumulative Na^+ excretion(meq)	6.98±1.18	7.88±1.88	17.70±2.71*	17.76±1.78*	21.24±5.21*
Cumulative K^+ excretion(meq)	16.12±3.86	14.72±1.91	23.60±4.36	19.73±4.60	17.78±1.16
Cumulative volume (ml)	76.25±14.34	63.94±4.44	119.56±19.08	89.31±13.56	98.5±16.92

mean±SE; n=4; experiment consisted of 1 hr control, 2 hr infusion, and 4 hr recovery *p=.01

The natriuretic responses to acute infusion were delayed in onset, and recovery was markedly prolonged in comparison to acute infusions with ANP (1-28). There were no significant changes in any cardiovascular parameters. These data extend previous findings that C-ANP receptor specific ligands have unique biological effects in vivo.

A 121 INTRACAROTID INFUSIONS OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN DEHYDRATED GOATS, Kerstin Olsson, Kristina Dahlborn, Katarina Nygren, Bengt E. Karlberg and Lea Eriksson, Swed. Univ. Agr. Sciences, Uppsala, Sweden, Univ. of Linköping, Sweden and College of Veterinary Medicine, Helsinki, Finland.

ANP from cardiac tissues is known to have vascular and renal effects, but ANP-like material and ANP receptors have been found also in the brain. Whether blood-borne ANP affects thirst and vasopressin release is controversial. ANP (1.5 or 4.7 μg/min) was infused via the carotid arteries for 40 min in goats water-deprived for 2 days. The low dose raised plasma ANP levels to 530 ± 106 pmol/l; i.e. above physiological levels. Water was offered after 35 min. The goats drank 2.9 ± 0.4 l (control; N = 7), 1.9 ±0.6 l (low dose; N = 5; NS), and 0.6 ±0.2 l (high dose; N = 5; P<0.01). Mean arterial blood pressure (MAP) fell only in response to the high dose (by 20 ±4 mm Hg; P<0.01). Drinking caused MAP to rise above pre-infusion levels (control and low dose; P<0.001), but only to approach pre-infusion levels during the high dose. Renal free water clearance became more negative during ANP infusions. Renal Na excretion increased about 3 times for the low dose (P< 0.01) and almost 10 times for the high dose (P<0.001). The results indicate that blood-borne ANP is not of physiological significance in the control of water balance, and that dehydration does not prevent an elevation of the renal Na excretion during ANP infusions.

A 122 RENAL EFFECTS OF ANF INFUSION IN CONSCIOUS MONKEYS, T.V. Peterson, B.A. Benjamin, C.H. Metzler and N.L. Hurst, Texas A&M University, College Station, Texas 77843, and University of California, San Francisco, CA 94143.

Experiments were performed to characterize the renal responses to atrial natriuretic factor (ANF) infusion in conscious *Macaca fascicularis* monkeys. The protocol consisted of a 30 min control period, 120 min of α-hANF infusion at 100 ng/kg/min and a 30 min postinfusion period with bladder urine being collected throughout as consecutive 10 min samples. ANF caused urine flow to increase from 0.34±0.05 to a peak value of 1.47±0.23 ml/min, absolute sodium excretion from 34±8 to 166±18 μEq/min and fractional sodium excretion from 1.21±0.23 to 5.74±1.03%. With regard to the pattern of the responses, significant increases in urine flow and sodium excretion did not begin occurring until 40 min into the infusion, reached the above peak effects at 70-80 min, and then began to decrease from these levels. Osmolar clearance and free water clearance increases were similar to this-i.e. slow onset and transient peak effects occurring at the midpoint of the infusion. Indices of renal hemodynamics, creatinine and para-aminohippurate clearances, were unchanged whereas arterial pressure decreased slightly and heart rate increased. These results show that the conscious monkey responds to 120 min of a 100 ng/kg/min infusion of ANF with an increase in renal excretion that is slow in onset with peak diuretic and natriuretic effects that are transient and not sustained throughout the duration of the infusion. (Supported by NIH Grants HL31987, HL01383, HL35879 and Texas Heart Grant 85G-027).

A 123 COMPLETE REVERSAL OF CICLOSPORINE-INDUCED ACUTE RENAL FAILURE (ARF) BY THE ATRIAL NATRIURETIC PEPTIDE (ANP), Schafferhans, K., Heidbreder E., Nothen, S., Heidland, A., Dept of Nephrology, University of Wuerzburg, FRG.

In recent studies it was demonstrated, that ANP prevents and reverses the acute ischemic renal failure. Moreover in uranyl-nitrate- and gentamicin-induced ARF a beneficial action of ANP was found. This study was designed to investigate the effects of ANP on ciclosporine-induced ARF. Methods and materials: Female Sprague-Dawley rats were used in this study. After right nephrectomy and clamping of the left renal artery(45 minutes), animals were housed in metabolic cages and permitted free access to water and standard rat chow. Over a period of 3 days, rats received ciclosporin(C) at 50 mg/kgBW i.p.). On day 4, animals were infused i.v. with ANP at 10 μg/kgBW/h vs. isotonic saline(NaCl). Inulinclearance(GFR), diuresis($\dot{V}$), absolute electrolyte excretion and arterial blood pressure were measured.
Results: In all rats, C induced a nonoliguric ARF. GFR had decreased significantly to one half of basal values. Subsequent ANP-infusion induced a highly significant increment of GFR to normal values($p<0.001$). Moreover, urinary volume exceeded diuresis of the toxic renal insult 8-fold, following ANP administration.
Discussion: In this study ANP was indeed capable of improving kidney function in C-induced-ARF. ANP has been cited to dilate mesangial and epithelial cells and antagonizes the Angiotensin II and Norepinephrine-induced constriction of renal microcirculation. Therefore, ANP is assumed to be responsible for increasing the ultrafiltration coefficient(K_f). This may be, at least in part, responsible for the beneficial action of ANP in toxic ARF.

A 124 THE RELATIONSHIP BETWEEN ATRIAL NATRIURETIC FACTOR AND RENAL FUNCTION IN HYPOTHYROID DOGS DURING VOLUME EXPANSION. Robert S. Zimmerman, John Ryan, Brooks S. Edwards, George G. Klee, Donald Zimmerman, Nigel A. Scott, John C. Burnett, Jr., Mayo Clinic, Rochester, MN

Atrial natriuretic factor (ANF) is decreased in hypothyroidism (HT), but the natriuretic response to volume expansion (VE) is intact. We hypothesized that ANF and sodium excretion ($U_{Na}V$) are dissociated in HT during VE. Five weeks following thyroidectomy (Tx), 8 anesthetized dogs underwent 10% body weight saline VE over 1 hr and measurements were made at 3.3%, 6.6%, and 10% VE. Following Tx, T_4 decreased from 1.4±0.1 to 0.4±0.1 ng/dl ($p<.05$) and ANF decreased from 95±9 to 47±8 pg/ml. ($p<.05$)

VE%	RAP mmHg	GFR ml/min	$U_{Na}V$ µEq/min	FE_{Na}%	ANF pg/ml
Control	1.0±0.5	26±2	39±13	1.0±0.4	42±8
3.3	3.3±0.6$^+$	31±2	323±64$^+$	7.1±1.3$^+$	59±13$^+$
6.6	4.6±0.7$^+$	29±2	521±96$^+$	12.4±2.2$^+$	107±30$^+$
10	6.1±1.0$^+$	27±2	577±125$^+$	14.1±2.9$^+$	109±30$^+$
REC	1.9±0.5	32±1$^+$	709±138$^+$	14.8±2.7$^+$	74±16

$^+p<.05$ compared to control, RAP, right atrial pressure; GFR, glomerular filtration rate; FE_{Na}, fractional excretion of sodium; REC, recovery. These results demonstrate that in HT, despite the decrease in basal levels, 1) ANF increases with increases in RAP, 2) the natriuresis of volume expansion is associated with increasing ANF, and 3) GFR is unchanged during VE despite increases in ANF.

Cardiovascular

A 125 CHARACTERIZATION OF MONOCLONAL ANTIBODIES THAT DISTINGUISH BETWEEN ANP 99-126 AND RING-CLEAVED (CYS_{105}-PHE_{106}) ANP_{99-126}, Kendra B. Eager, Patricia M. Deneck, Ophelia W. Hadjilambris and Jon A. Norman, The Squibb Institute for Medical Research, Princeton, N. J. 08543. We have identified several monoclonal antibodies to Atrial Natriuretic Peptide (ANP) and have examined their reactivity to ANP fragments. BALB/c mice were immunized with a conjugate prepared by glutaraldehyde crosslinking of rat ANP (99-126) to keyhole limpet hemocyanin (KLH). Spleen cells from responding animals were fused with the Sp2/0-Ag14 myeloma line and the resulting hybrids were screened for antibody reactivity to ANP_{99-126} by enzyme-linked immunosorbent assay (ELISA). Twenty-six hybrid clones secreting anti-ANP antibody were selected from greater than one thousand clones. These clones have been subcloned and evaluated for the class of antibody produced and their specificity to ANP. Several hybridomas have been further characterized to determine the binding epitope on ANP by using peptide fragments of ANP in an ELISA prodedure. Atriopeptin I was not recognized by any of the MAbs whereas polyclonal antisera directed to ANP can bind to this peptide. The relative binding of the monoclonal antibodies to molar equivalents of various peptides was determined. MAb 45-16 bound ring-cleaved (Cys_{105}-Phe_{106}) ANP_{99-126} > ANP_{99-126} > $AP_{101-126}$ > APIII. MAb 45-29 bound ANP_{99-126} ≥ $AP_{101-126}$ > ring-cleaved (Cys_{105}-Phe_{106}) ANP_{99-126} > APIII. MAb 4-30 bound ANP_{99-126} > $AP_{101-126}$ > APIII but only negligibly reacted with ring-cleaved (Cys_{105}-Phe_{106}) ANP_{99-126}. However, a rabbit polyclonal antibody bound greatest to $ANP_{101-126}$ ≥ ANP_{99-126} ≥ ring-cleaved (Cys_{105}-Phe_{106}) ANP_{99-126} > APIII ≥ API.

A 126 LOCALIZATION OF ANP IN THE VENTRICLES OF SPONTANEOUSLY HYPERTENSIVE RAT. J. Gu, MD, PhD; L. Gonzalez-Lavin, MD; N. Kulatilake, MB, FRCS; S. Cull, AAS; M. D'Andrea, BS; R. Fiscus, PhD. Deborah Research Institute, Browns Mills, NJ 08015

The occurrence and distribution of α-atrial natriuretic peptide (α-ANP) immunoreactivity in the ventricles of spontaneously hypertensive rats were studied with the methods of immunocytochemistry at both the light and the electron microscopic levels. The ANP immunoreactivity was found in the specific granules in the cytoplasm of the cardiocytes in the myocardium, and only sparsely in the endocardium and the epicardium. The abundant specific granules are more evenly distributed in the cytoplasm of the myocytes in the ventricles than those found in the atria, otherwise the ANP-containing granules in the ventricles and the atria are similar in size, shape and ANP immunoreactive content. The abundance of the ANP immunoreactivity in the left ventricle is higher than that in the right and appears to increase with the severity of hypertension and with increased age of the animals. The overall content of ANP in the atria of hypertensive rats seems to be decreased when compared with the age-matched normotensive rats. The present findings indicate that ventricles may become the major source for ANP synthesis and release during hypertension, and may play important roles in cardiac endocrine pathology and cardiac hypertrophy.

A 127 IMMUNOREACTIVE ATRIAL NATRIURETIC PEPTIDE IN VENTRICLES, ATRIA, HYPOTHALAMUS AND PLASMA OF GENETICALLY HYPERTENSIVE RATS, Heikki Ruskoaho and Juhani Leppäluoto, University of Oulu, SF-90220 Oulu, Finland.

To evaluate the role of extra-atrial atrial natriuretic peptide (ANP) in volume and blood pressure regulation, the plasma, atrial, ventricular and hypothalamic levels of immunoreactive atrial natriuretic peptide (IR-ANP) were measured simultaneously in the spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats at the age of 2, 6 and 12 months. Plasma IR-ANP in the 12-months-old, conscious SHR was significantly higher than that of the WKY rats, while no differences between the strains in plasma IR-ANP levels were found in younger rats. The older SHR had attenuated ANP release to acute volume expansion with saline (1.1 ml/100g b.wt.) as shown by the shift of the ANP versus right atrial pressure curve to the right. The total atrial IR-ANP content (ug/atria) was consistently lower in the SHR compared to the WKY rats, whereas the hypothalamic IR-ANP concentration was significantly increased in the SHR compared to that of WKY rats. In both ventricles, IR-ANP concentrations and contents increased with increasing age in WKY and SHR rats, but the ventricular levels of ANP were reduced in ventricles of the SHR heart compared with normotensive controls. The depletion of total ventricular IR-ANP was greatest in SHR with greatest ventricular hypertrophy and coincided with the attenuated ANP release to acute volume load. The increase of left but not right ventricular weight occurring secondary to six weeks minoxidil treatment was accompanied by higher ANP concentration in both strains. Thus, ventricular and hypothalamic, as well as atrial ANP, respond to increased pressure overload. Our results suggest that chronic stimulation of ANP release from ventricles is associated with depleted stores of atrial natriuretic peptide from both ventricles and reduced response to acute volume load. Our findings that the increase of ventricular weight occurring with age or secondary to a hypertrophic stimulus is associated with increased ventricular ANP and the decreased ventricular ANP concentration in SHR with the most severe ventricular hypertrophy suggest that ANP in ventricles is not only synthetized for circulation as a hormone but might locally have an influence on the development of ventricular hypertrophy.

Experimental Pathophysiology

A 128 PRESENCE AND DISTRIBUTION OF CARDIAC ANF IN HUMANS WITH CONGESTIVE HEART FAILURE. Brooks S. Edwards, Irvin Goldenberg, Marc Pritzker, Robert W. Emery, Lester E. Wold, Margaret M. Redfield, John C. Burnett, Jr., Mayo Clinic, Rochester, and Minneapolis Heart Institute, Minneapolis, MN

We have previously reported the presence of immunoreactive ANF granules in ventricular myocardium of hamsters with congestive heart failure (CHF) as well as humans with CHF undergoing diagnostic right ventricular endomyocardial biopsy. The extent and distribution of ANF within the ventricular myocardium of the human with CHF is, however, unknown. To extend our previous biopsy studies, five intact human hearts from subjects with ischemic cardiomyopathy were obtained at the time of orthotopic cardiac transplantation and full thickness ventricular and atrial sections examined. ANF granularity was assessed utilizing a two-stage immunohistochemical technique with specific antibody to alpha-human ANF. Each section was reviewed and graded by two independent observers. Full thickness right and left atrial sections revealed extensive ANF granularity in all hearts. ANF was detected within both right and left ventricular tissue of all specimens. Examination of ventricular myocardium revealed a gradient for granules with granularity greatest in the subendocardial regions of the ventricle. Ventricular granularity appeared greater in the left as compared to the right ventricle. Atrial granularity significantly exceeded ventricular granularity. In summary, this study for the first time reports the presence and distribution of atrial and ventricular ANF immunoreactive granularity in the hearts of humans with end-stage congestive heart failure.

A 129 IMPAIRMENT OF THE ATRIAL NATRIURETIC FACTOR (ANF) SYSTEM IN PATIENTS WITH CIRRHOSIS OF THE LIVER. Alexander L. Gerbes, Rainer Arendt, Dieter Jüngst, and Gustav Paumgartner, Klinikum Grosshadern, Univ. of Munich, Federal Republic of Germany.

Deficiency of natriuretic factors has been postulated in patients with cirrhosis and retention of sodium and water. We demonstrated that ANF plasma levels in cirrhotics with ascites are not lower than in cirrhotics without ascites (N Engl J Med 313:1609,1985). Aim of the present study was to investigate the functional status of the ANF system in compensated and decompensated cirrhosis. Methods. ANF was determined in extracted plasma samples as described before (FEBS Lett 198:57,1985). For infusion, synthetic ANF_{99-126} (Bissendorf,West Germany) was used. Results. 1) No significant differences of ANF plasma levels were observed between 41 cirrhotics and 35 healthy controls (9±1 vs.8±1 fmol/ml). 2) ANF stimulation following central volume loading by 1 hour water immersion was significantly blunted in 10 cirrhotics with ascites (increase of plasma ANF by 46±18%) as compared to 11 cirrhotics without ascites (104±16% increase) and to 25 controls (117±29% increase) at comparable central volume stimulation. Increases of natriuresis following immersion were not significant in cirrhotics with (43±19 umol/min) and without ascites (75±43 umol/min), but in controls (146±38 umol/min). 3) Infusion of ANF (50 ng/kg/min) for 30 min increased diuresis by 1.8±0.3 ml/min and natriuresis by 105±19 umol/min in 4 cirrhotics with ascites as compared to 6.8±3.6 ml/min and 195±93 umol/min in 3 cirrhotics without ascites. Conclusions. Volume stimulated release of ANF as well as renal response to ANF seem to be blunted in cirrhotics with ascites. These impairments of the ANF system might contribute to pathophysiology of ascites formation in cirrhosis of the liver.

A 130 COUNTERACTION OF THE ATRIAL NATRIURETIC FACTOR (ANF) AND THE RENIN-ALDOSTERONE SYSTEM IN VOLUME REGULATION OF LIVER CIRRHOSIS. Alexander L. Gerbes, Heinrich Wernze, Rainer Arendt, Tilmann Sauerbruch and Gustav Paumgartner. Depts. of Medicine, Univ. of Munich and Würzburg, Federal Republic of Germany.

The role of the atrial natriuretic factor and of the main sodium retaining principle, the renin-aldosterone system in acute volume regulation of decompensated and compensated human cirrhosis was investigated. 25 healthy controls (Co) and 21 cirrhotics (Ci), 11 without (-A) and 10 with ascites (±A) underwent 1 hour head-out water immersion resulting in central volume stimulation. Immersion prompted a highly significant increase of plasma ANF from 8.5±1.3 to 16.5±2.6 fmol/ml in Ci-A, comparable to the stimulation in Co. In Ci+A,ANF increase was blunted (7.7±1.3 to 11.4±2.3 fmol/ml). Comparable decreases of plasma renin activity (PRA, ng AI/ml/h) and plasma aldosterone concentration (PAC, ng/100 ml) were seen in Co (PRA:5.3±0.9 to 2.4±0.3, PAC:13.0±1.7 to 6.5±0.8), Ci-A (PRA:8.6±2.8 to 4.8±1.8; PAC:38.1±11.3 to 26.6±9.0) and Ci+A (PRA:28.9±9.4 to 17.3±6.8;PAC:63.9±19.9 to 49.1±19.3). Immersion induced a more pronounced natriuresis and diuresis in Co than in Ci, particularly than in Ci+A. Neither ANF, nor PRA or PAC alone were correlated to basal or stimulated renal response. However, baseline and stimulated ratios of ANF/PRA and more so, of ANF/PAC correlated significantly to the corresponding natriuresis and diuresis in cirrhotics, but less in controls. Conclusions. In patients with cirrhosis and ascites ANF response to acute volume stimulation is blunted. Counteraction of ANF with the renin-aldosterone system seems to determine urinary volume and sodium excretion. It is the ANF/Aldosterone ratio rather than the single parameters that influences both, basal and volume stimulated natriuresis and diuresis in cirrhosis.

A 131 **PLASMA CONCENTRATIONS OF ATRIAL NATRIURETIC PEPTIDE IN A MODEL OF ACUTE HEART FAILURE** Christian Hall, Olav Hevrøy, Nils E. Kløw and Otto A. Smiseth. Institute for Surgical Research, University of Oslo and Dept of Physiology, University of Tromsø, Norway.

Plasma concentrations of atrial natriuretic peptide (ANP) is typically increased in chronic congestive heart failure. We studied ANP levels in the plasma of 6 anaesthetized labrador dogs after the induction of acute ischemic left ventricular heart failure. Failure was induced by repeated embolization of the left coronary artery with 55 µ plastic microspheres. Heart rate (HR), mean arterial pressure (MAP), right atrial pressure (RAP), left ventricular end-diastolic pressure (LVEDP) and ANP plasma concentrations (ANPc) were measured at baseline and 10 (Failure 1) and 60 (Failure 2) minutes after the last dose of embolizing solution. Results:

Variable	Baseline		Failure 1	Failure 2	N=6
HR(beats/min)	143(14)		145(18)	147(16)	Mean(SD)
MAP(mmHg)	114(14)	*	96(15)	91(18)	
RAP(mmHg)	2.4(2.3)	*	6.3(2.8)	6.0(2.7)	
LVEDP(mmHg)	4.7(1.8)	*	19.4(3.8)	20.9(3.4)	
ANPc(pmol/l)	21.0(8.1)	*	26.7(10.4)	28.4(16.2)	*:p<0.05.

Although LVEDP and RAP increased substantially after coronary embolization there was only a 27 % increase in ANPc. ANP does not seem to play an important role in the response of the organism to acute heart failure.

A 132 EFFECT OF DIFFERENT DOSING REGIMENS ON THE RESPONSES TO CHRONIC INCREASES IN PLASMA ATRIAL NATRIURETIC PEPTIDE (ANP) CONCENTRATION. C.H. Metzler, D.A. Roberson, R.D. Krandt, R.M. Scarborough, L.C. Gregory, G.A. McEnroe and J.A. Lewicki, Univ. of Calif., School of Medicine, San Francisco, CA and California Biotechnology, Inc., Mountain View, CA.

A number of reports have shown that chronic administration of ANP to both animals and man in both normal and pathophysiologic conditions have only transient effects on sodium excretion and blood pressure. To investigate the mechanisms responsible for this "escape", plasma atrial peptide concentration was increased by infusing either rANP 1-28 or an ANP analogue ([des(18-22)]rANP(4-23)-NH_2, desQ) which binds to the ANP clearance receptor and decreases the clearance of endogenous atrial peptide from the circulation (Maack et al., Science, In Press). Both peptides were infused into conscious dogs using either a continuous infusion protocol or a pulsed infusion protocol in which the same daily dose was administered in four, equally spaced 1 hour infusions. Continuous infusion of both rANP (72 ug/kg/day) and desQ (432 ug/kg/day) resulted in transient natriuresis on the first day of administration (29.0 mEq and 31.6 mEq above control, respectively) and "escape" on the second day (-19.1 and -7.0 mEq). Pulsed infusion of both rANP and desQ resulted in progressive natriuresis that persisted for the entire three day infusion period (55.1 mEq and 159.9 mEq over three days, respectively). Similar results were obtained for daily urine volume. No consistent changes in urinary potassium excretion or blood pressure were observed. These results demonstrate that pulsed administration of atrial peptides can lead to progressive and sustained natriuresis and suggest that ANP may be clinically useful when used in this manner.