closely related to SRV-1 by nucleic acid hybridization studies and serological analyses (*3*). The DNA sequence of molecularly cloned SRV-2 reveals a high degree of homology to SRV-1; the greatest sequence variation is in the amino terminal domain of the *env* genes (*40*). Genetically engineered recombinant viruses, made by exchanging portions of these related viruses, will be useful in determining which sequence of the genome of SRV-2 is associated with retroperitoneal fibromatosis. Novel vaccine strategies that use, for example, subunit envelope proteins of SRV-1 or SRV-2 expressed in recombinant microorganisms may help control SAIDS in infected primates.

Note added in proof: The location of the *gag* phosphoprotein of SRV-1 in Fig. 1 is based on the published amino terminal sequence of the MPMV phosphoprotein (pp18) (*27*). The amino terminal sequence of the phosphoprotein of the related SAIDS retrovirus D/W isolate, from the Washington Regional Primate Research Center (*27*), corresponds to *gag* position 107 in Fig. 1. Thus, SRV-1 contains DNA sequences encoding the published amino termini of both MPMV and SAIDS retrovirus D/W.

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Inhibition of Vasopressin Action by Atrial Natriuretic Factor

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Atrial natriuretic factor results in diuresis in animals and humans, perhaps because atrial natriuretic factor increases renal blood flow. The possibility that this diuresis is due to direct inhibition of renal tubular epithelial water transport was examined in rabbit collecting tubules perfused in vitro. Atriopeptin III inhibition of the hydraulic conductivity response to the hormone arginine vasopressin but not to either 3′5′-cyclic adenosine monophosphate or forskolin was found. These results suggest that atriopeptin III acts proximal to cyclic adenosine monophosphate formation to directly affect vasopressin-stimulated water transport in the mammalian nephron. They also suggest a potential role for inhibition by atrial natriuretic factor of the renal response to arginine vasopressin as a contributor to a diuretic state.

MAMMALIAN ATRIA CONTAIN SECRETORY GRANULES. In response to atrial distension, these granules release a group of closely related 21- to 26-amino-acid peptides (*1–3*). Collectively referred to as atrial natriuretic factor (ANF), these peptides exert potent vasodilatory and diuretic effects in animals and humans (*1–7*). The mechanism of the diuretic action of ANF has not yet been established (*1–3*). Evidence obtained in vivo suggests that ANF increases the glomerular filtration rate and that the filtered load of salt and water is responsible for the diuresis (*2–4*). However, ANF reduces systemic arterial pressure, has a variable effect on renal blood flow and vascular resistance, and increases solute excretion (*1–7*).The multiplicity of systemic and intrarenal effects exerted by ANF in vivo renders determination of a direct renal epithelial cell effect on salt and water transport difficult. Whether tubular effects also contribute to the diuresis of ANF is unclear. To date, there is no evidence that ANF directly inhibits intact renal tubular epithelial cell salt and water reabsorption (*1–3, 7*). Our studies were designed to determine whether ANF exerts a direct effect on water transport in renal collecting tubules.

Individual rabbit renal cortical collecting tubules were obtained by microdissection and perfused in vitro by slight modifications of the method developed by Burg and others (*8–10*). Tubules were bathed in a solution of NaCl, 115; $MgSO_4$, 1.2; $CaCl_2$, 1.0; KCl, 5.0; sodium acetate, 10; NaH_2PO_4, 1.2; $NaHCO_3$, 25; and dextrose, 5.5 (all in millimoles per liter). Bath fluid of *p*H 7.40 and 25°C was completely changed every 3 to 4 minutes (*10*). The composition of the perfusion fluid was the same as that of the bathing fluid except that the final concentration of NaCl was reduced to 50 mmol/liter. Perfusion fluid also contained sufficient [^{14}C]inulin (New England Nuclear) to result in collected fluid count per minute at least 10- to 15-fold above background. The tubule was visually inspected at 1- to 3-minute intervals throughout the study. Hydraulic conductivity (measured in cm atm^{-1} sec^{-1} × 10^{-7}) was calculated from the formula derived by Al-Zahid *et al.* (*11*).

Collecting tubules were allowed to equilibrate at 25°C for 4 hours (*10*). Tubules were perfused at 10 to 12 nl/min by adjusting hydrostatic pressure of the fluid entering the perfusion pipette. Tubular length was comparable in all groups of studies. In all studies each tubule served as its own control. In each tubule four or five collections were obtained for measurement of hydraulic con-

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ductivity immediately after the addition of arginine vasopressin (AVP) (Sigma, St. Louis) to the bathing fluid. After these collections were obtained, atriopeptin III (Peninsula Laboratory) was added to the bathing fluid containing the AVP, and four collections for hydraulic conductivity measurement were obtained. After these collections, atriopeptin III was removed from the bathing fluid and four collections for hydraulic conductivity measurement were obtained in the presence of AVP alone.

Initial studies examined the effect of $2 \times 10^{-9} M$ atriopeptin III on hydraulic conductivity response to AVP (100 μU/ml, $n = 7$). Atriopeptin III decreased AVP-stimulated hydraulic conductivity in each tubule and the mean decrement in hydraulic conductivity was 30 percent (174 ± 31 before, and 121 ± 31 cm atm^{-1} 10^{-7} after ANF, $P < 0.025$, paired t test). This inhibition was not reversible upon removal of atriopeptin III (hydraulic conductivity 109 ± 36 cm atm^{-1} sec^{-1} 10^{-7}). We next examined the effects of lower concentrations of atriopeptin III ($10^{-9} M$) on hydraulic conductivity stimulated by AVP (50 μU/ml) ($n = 10$). In these studies (Fig. 1), atriopeptin III reversibly reduced AVP-stimulated hydraulic conductivity by 20 percent ($P > 0.05$). Mean hydraulic conductivity before, during, and after atriopeptin III in these AVP-treated tubules was 105 ± 23, 84 ± 21, and 100 ± 24 cm atm^{-1} sec^{-1} 10^{-7}, respectively. By contrast, no decrease in hydraulic conductivity was observed in time-control AVP-treated tubules ($n = 6$) exposed only to the carrier solution of atriopeptin III (hydraulic conductivity 115 ± 17, 129 ± 9, and 132 ± 11 cm atm^{-1} sec^{-1} 10^{-7} before, during, and after carrier solution respectively; NS).

Intracellular cyclic adenosine monophosphate (cAMP) is an acknowledged mediator of the hydroosmotic response to AVP. To determine if atriopeptin III inhibits vasopressin action by acting prior to or after cAMP formation, we measured the effect of $10^{-9} M$ atriopeptin III on hydraulic conductivity stimulated by chlorphenylthiocyclic 3′5′-adenosine monophosphate (ClPheS-cAMP, Sigma, $10^{-4} M$). In these experiments ($n = 5$), hydraulic conductivity was the same in the presence and in the absence of atriopeptin III (153 ± 15 before, 155 ± 10 during, and 159 ± 13 cm atm^{-1} sec^{-1} 10^{-7} after atriopeptin III, respectively, NS). These results suggest that atriopeptin III inhibits AVP-stimulated cAMP formation.

Hormonal stimulation of cellular cAMP formation requires the interaction of at least three distinct cell membrane protein components. These components include a specific hormone receptor, guanine nucleotide regulatory proteins capable of modulating receptor input, and a catalytic subunit, which converts the substrate magnesium adenosine triphosphate to cAMP. Forskolin is a readily soluble diterpene that increases intracellular cAMP by stimulating the catalytic subunit of adenylate cyclase (*9, 12*). To determine if the inhibitory effect of atriopeptin III on AVP action occurs at or before the catalytic subunit, we examined the effect of atriopeptin III ($10^{-9} M$) on forskolin-stimulated hydraulic conductivity. In these experiments ($n = 8$), forskolin ($10^{-5} M$)-stimulated hydraulic conductivity was comparable before, during, and after atriopeptin III (168 ± 10, 163 ± 12, and 193 ± 20 cm atm^{-1} sec^{-1} 10^{-7}, respectively, NS). These results, together with our cAMP studies, suggest that atriopeptin III inhibits AVP by acting proximal to the catalytic subunit of adenylate cyclase. Recently, ANF stimulation of cyclic guanosine monophosphate (cGMP) has been described in several tissues including renal collecting tubular cells (*13–15*). We therefore examined the effect of cGMP on AVP-stimulated hydraulic conductivity. In these experiments ($n = 5$), AVP-stimulated hydraulic conductivity was not significantly decreased by $10^{-4} M$ cGMP (89 ± 14, 99 ± 16, and 83 ± 14 cm atm^{-1} sec^{-1} 10^{-7} before, during, and after, respectively).

Most evidence suggests that an increase in glomerular filtration rate and other renal hemodynamic effects are responsible for the diuretic effect of ANF (*1–6*). However, high-affinity specific binding for ANF has been identified in mammalian renal cortical membranes, and autoradiographic studies demonstrate renal tubular uptake, suggesting the possibility of renal tubular ANF receptors (*16, 17*). Recently, ANF inhibition of solute transport in apical brush border membrane vesicles from renal epithelial cells was demonstrated (*7*). Our results demonstrate atriopeptin III inhibition of the water permeability response to AVP in intact mammalian collecting tubules. Preliminary studies undertaken in toad urinary bladder also demonstrate an effect of ANF to inhibit the hydroosmotic response to arginine vasotocin, a peptide closely related to AVP (*18*). The inhibition of AVP by atriopeptin III can contribute to the diuresis and high free-water clearance from the kidney that follows ANF administration in vivo (*6*).

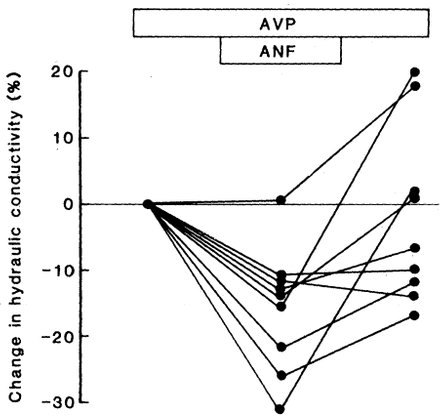

Fig. 1. Effect of ANF (atriopeptin III, $10^{-9} M$) on AUP-stimulated hydraulic conductivity.

We used pharmacological probes which suggest that atriopeptin III inhibition of the hydraulic conductivity response to AVP occur in the basolateral membrane at a site or sites proximal to cAMP formation. Two such sites include the vasopressin receptor and the guanine nucleotide coupling unit. Observations by others demonstrate that ANF stimulates guanylate cyclase and cGMP formation in AVP-responsive epithelial cells (*13*). We did not find, however, that exogenous cGMP alters AVP-stimulated water permeability. Although further studies will be required to delineate the precise site and mechanism of inhibition, our studies show a pre-cAMP inhibitory effect of ANF on AVP-stimulated transepithelial osmotic water transport in the intact mammalian nephron. Taken in the context of recent experiments showing that AVP stimulates release of ANF (*19*), our studies suggest the existence of an AVP-ANF negative-feedback endocrine loop regulating renal water homeostasis.

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