work on the physiological role and tissue distribution of the VGF8a sequence is required, our results suggest that VGF8a is predominantly expressed in neuronal cells and tissues.

We cannot rule out a low level of expression of the VGF8a mRNA in untreated PC12 cells. Nevertheless, our data suggest a rapid activation of the VGF8a gene by NGF. Within 5 hours of the addition of NGF to PC12 cells, the VGF8a sequence was induced to maximal levels, comparable to those of actin mRNA, and this level of expression is only diminished two- to threefold in cultures treated with NGF for 2 weeks. On the basis of this amount of expression, the protein encoded by the VGF8a mRNA would be expected to represent a major product in NGF-treated cells. We have no explanation for the inability to detect such a protein in previous studies (*6, 7*). The polypeptide encoded by the VGF8a mRNA sequence may have an unusual isoelectric point, or the polypeptide may represent a precursor that is modified or processed such that the final products would not appear in the gel pattern.

Initial sequence analysis of the VGF8a clone does not indicate any significant sequence homology with sequences in the National Sequence Data Bank, and direct hybridization studies with oncogene probes have been negative. Hybridization of the VGF8a clone to Eco RI- and Hind III-digested genomic rat DNA indicates that this portion of the gene is in the single-copy range and is contained within fragments of less than 6 kilobases. It is conceivable that the VGF8a-related mRNA encodes one of the microtubule-associated proteins induced by NGF in PC12 cells, as described by Biocca *et al.* (*15*).

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Vasopressin-Stimulated Release of Atriopeptin: Endocrine Antagonists in Fluid Homeostasis

Abstract. *Administration of pharmacological doses of arginine-vasopressin, related peptides, and other pressor agents induced a profound release of atriopeptin immunoreactivity into the circulation. The stimulated release of atriopeptin apparently was related to increased arterial blood pressure. Neither the nonpressor vasopressin analog 1-deamino-D-Arg^8-vasopressin nor arginine-vasopressin in the presence of a specific pressor antagonist caused atriopeptin to be released into the circulation. Urine output was correlated with the level of atriopeptin released. Physiological levels of arginine-vasopressin suppress diuresis and produced vasoconstriction. Pharmacological levels of the hormone stimulated the cardiac endocrine system to release atriopeptin, which may cause diuresis and vasodilation to physiologically antagonize the effects of vasopressin.*

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Mammalian atrial myocytes contain peptides [atriopeptins (AP's)] that have potent natriuretic, diuretic, and vascular smooth muscle relaxant properties (*1*). Support for the hypothesis that AP's are involved in hormonal regulation of the electrolyte and extracellular fluid balance requires characterization both of the release of AP's from the heart and of their subsequent effects on target organs, including the kidneys and vasculature. Current efforts are focused on the direct demonstration of the endocrine nature of AP's. The release of low molecular weight AP's into the coronary venous circulation by isolated, perfused mammalian hearts (*2*) is facilitated by atrial stretching (*3*). Distention of the left atrium and expansion of intravascular volume in conscious dogs produce natriuresis and diuresis (*4*), possibly because of the release of AP's into the blood. Conversely, removal of the right atrial appendage decreases the natriuretic and diuretic response to acute hypervolemia in the rat (*5*). Muscarinic and adrenergic agonists as well as the intrinsic hormone arginine-vasopressin (AVP) have been reported to induce the release of AP's from isolated atria; however, appropriate tissue controls were lacking in this study (*6*). Recent radioimmunoassays indicate that rat plasma contains immunoreactive AP's (*7, 8*). Profound acute expansion of intravascular volume in rats results in the transient appearance in the circulation of an unidentified material that is immunologically related to AP's

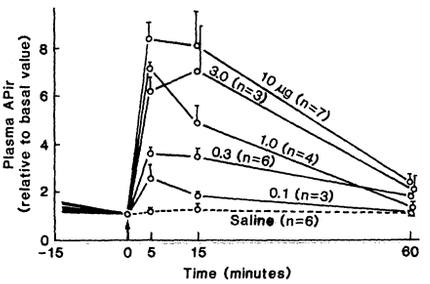

Fig. 1. Dose-dependent stimulation of AP release into the circulation by dAVP. Male Sprague-Dawley rats were anesthetized intraperitoneally (0.5 ml of 7 percent chloral hydrate per 100 g of body weight) and the jugular vein and carotid artery were cannulated (PE 50 tubing). After a 20-minute equilibration period, arterial blood samples were collected 15 minutes and immediately prior to, as well as 5, 15, and 60 minutes after, intravenous administration of various doses of dAVP. Blood samples were collected into one-tenth of a volume of 0.11*M* sodium citrate and centrifuged, and plasma was immediately frozen and stored at −70°C until being assayed for APir by radioimmunoassay (*8, 18*). The blood removed (0.3 ml) was replaced with an equivalent volume of saline. Values are means ± standard errors for *n* animals and are expressed relative to values at zero time. Mean basal plasma APir was 0.85 ± 0.07 ng/ml.

and that migrates chromatographically in their vicinity (*3*). We report here that AVP, related compounds, and pressor agents induce profound and sustained release of AP immunoreactivity (APir) into the circulation.

Administration of 1-deamino-Arg^8-vasopressin (dAVP) (0.1 to 10 μg) produced a dose-dependent increase in plasma APir that peaked 5 to 15 minutes after the injection and returned to the basal value (0.85 ± 0.07 ng/ml) within 60 minutes (Fig. 1). Neither an equivalent 0.1-ml bolus of saline nor the periodic replacement, with saline, of blood samples taken affected the basal concentration of APir (Fig. 1). Two low molecular weight AP's account for the major portion of this hormone-stimulated APir (*8*). Although acute changes in blood pressure were maximal after the administration of 1 μg of dAVP, APir release continued to increase at doses up to 10 μg. Doses of dAVP in excess of 10 μg were not tested.

Table 1. Effects of vasoconstrictors on urine output. Rats were treated as described in the legends to Figs. 1 and 3. All drugs were dissolved in 0.1 ml of normal saline and given as a bolus. Control rats were treated with 0.1 ml of saline alone. Bladders were catheterized and urine was collected for the 15 minutes before and the 60 minutes after drug administration. Values are means ± standard error for four rats in each treatment group.

Treatment	Basal output (milliliters per 15 minutes)	Stimulated output (milliliters per 60 minutes)
Control	0.10 ± 0.04	0.51 ± 0.17
dAVP (1 μg)	0.07 ± 0.04	1.80 ± 0.23
AVP (1 μg)	0.13 ± 0.06	1.22 ± 0.33
Phenylephrine (3 μg)	0.11 ± 0.05	0.42 ± 0.13
Angiotensin II (1 μg)	0.08 ± 0.05	0.47 ± 0.32

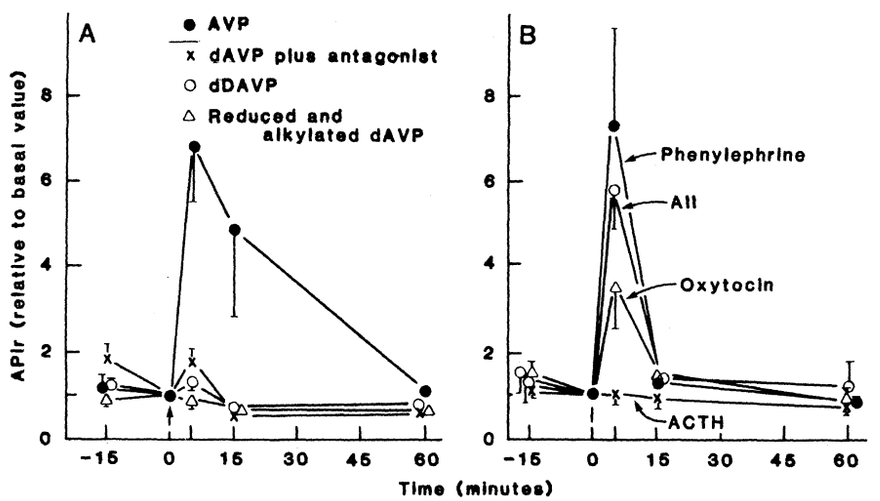

Fig. 2. Comparative effect of vasopressin analogs and pressor agents on plasma AP. Animals were treated as described in the legend to Fig. 1. (A) Effects of treatment with 3 μg of AVP, 3 μg of dDAVP, 1 μg of dAVP plus 100 μg of [1-(β-mercapto-β,β-cyclopentamethylene proprionic acid),2-(*O*-methyl)tyrosine]-Arg^8-vasopressin, or 3 μg of reduced and alkylated dAVP. (B) Effects of treatment with 30 μg of phenylephrine, 10 μg of angiotensin II (AII), 10 μg of oxytocin, or 10 μg of adrenocorticotropic hormone (ACTH). Values are means ± standard errors for three rats for each treatment group.

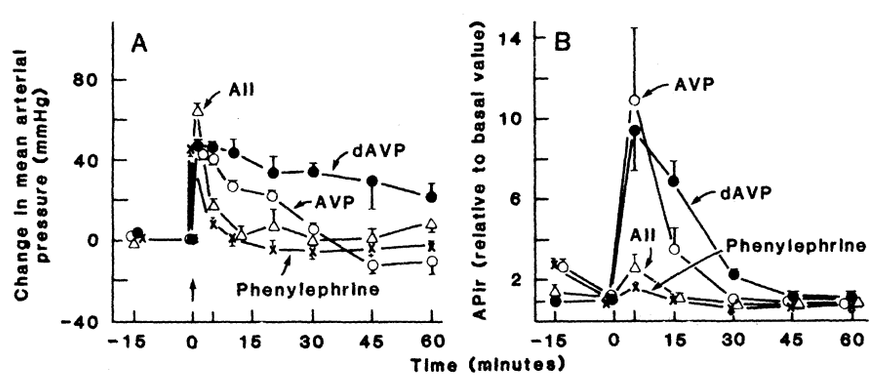

Fig. 3. Relation between changes in blood pressure and plasma AP levels. Animals were treated as described in the legend to Fig. 1 with the following exceptions: carotid arterial pressure was monitored with a Beckman R511 physiograph equipped with a type 9853H pressure transducer. Blood samples were obtained from the femoral artery at −15, 0, 5, 15, 30, 45, and 60 minutes. Urine output was monitored by bladder catheterization. (A) Blood pressure changes and (B) APir after treatment with 1 μg of dAVP, 1 μg of AVP, 3 μg of phenylephrine, or 1 μg of angiotensin II. Values are means ± standard errors for four rats in each treatment group.

In an attempt to elucidate the mechanism of action of dAVP-induced release of APir, we tested AVP and several related peptides (Fig. 2A). AVP induced dose-dependent increases in plasma APir comparable to those produced by similar doses of dAVP (results are shown for the 3-μg dose only). Disulfide reduction and alkylation (*9*) of dAVP largely abolished its pressor and diuretic activities and its effects on AP release (Fig. 2A). Administration of an antagonist to the pressor activity of AVP, [1-(β-mercapto-β,β-cyclopentamethylene proprionic acid),2-(*O*-methyl)tyrosine]-Arg^8-vasopressin (Peninsula) (*10*), in a ratio of antagonist to dAVP of 100 to 1, prevented the increase in APir (Fig. 2A). Treatment with antagonist alone had no effect on the basal concentration of AP in plasma. A synthetic vasopressin analog with marked antidiuretic activity but virtually devoid of pressor activity, 1-deamino-D-Arg^8-vasopressin (dDAVP; Ferring Pharmaceuticals) (*11*), did not induce APir release (Fig. 2A). In contrast, oxytocin, a neurohypophyseal nonapeptide with marked homology to and with less than 10 percent of the pressor activity of AVP, also elicited a dose-dependent release of APir (results of the 10-μg dose are shown in Fig. 2B). Several other agents that affect natriuresis (*12*) or blood pressure (*13*) were also tested for their ability to induce APir release (Fig. 2B). Adrenocorticotropin was without effect. Phenylephrine, an α-adrenergic agonist, and angiotensin II, a potent vasoconstrictor, induced release of APir in a dose-dependent manner.

To determine whether increases in blood pressure mediated the observed AP release, we treated rats with the vasoconstrictors dAVP, AVP, phenylephrine, and angiotensin II at doses that produced comparable acute changes in peak blood pressure. All compounds caused an acute increase in mean arterial pressure of 40 to 60 mmHg; however, the duration of the increase differed for each compound (dAVP> AVP > angiotensin II ≥ phenylephrine) (Fig. 3A). Peak levels of APir in plasma appeared within 5 minutes of administration of the agents (Fig. 3B). The time course of release of APir into the plasma was correlated with the duration of the hypertension produced. Bolus administration of phenylephrine or angiotensin II induced very transient increases in both arterial pressure and APir release (Fig. 3). In con-

trast, bolus treatment with dAVP and AVP produced a more prolonged increase in blood pressure and APir release. In addition, continuous infusion of phenylephrine (10 μg/min) produced a sustained 15-mmHg elevation in mean arterial pressure and a three- to fourfold elevation in plasma APir. Both of these effects were reversible upon termination of the infusion. The dependence of APir release on changes in arterial pressure is consistent with the observation that neither the nonpressor analog dDAVP nor AVP in the presence of its antipressor antagonist caused APir release.

Changes in systemic pressure may not be the only factor involved in the release of APir. There were significant differences in the initial amount of APir released in response to matched increases in mean arterial pressure. These differences may be related to regional changes in pressure or blood flow, especially those affecting atrial volume and wall tension. Alternatively, dAVP and AVP could be acting through a parallel but pressure-independent mechanism to augment APir release.

Average urine output was relatively stable in the control animals during the experiment (Table 1). Treatment with dAVP and AVP produced significant diuresis, while phenylephrine and angiotensin II did not (Table 1), suggesting that a sustained release of APir may be necessary to effect an increase in diuresis. Natriuresis has been shown to occur after administration of vasopressin (*14, 15*); however, the mechanism underlying this effect is not known. A possible explanation is that a sustained elevation in blood pressure causes the diuresis. Depression of proximal tubular reabsorption of sodium (*14*) and activity of a subtype of vasopressin receptor specific for the natriuretic activity have also been postulated (*16*). The observed dAVP-induced release of APir in vivo provides a further explanation of the natriuretic effects of vasopressin and related peptides.

In conclusion, we have shown that the release of endogenous APir in intact animals can be induced by a naturally occurring peptide hormone, arginine-vasopressin. In a recent study it was demonstrated that infusion of APIII lowers elevated arginine-vasopressin in dehydrated or hemorrhaged animals (*17*). This suggests the existence of a negative-feedback endocrine loop whereby arginine-vasopressin stimulates AP release, which in turn suppresses arginine-vasopressin release. These opposing hormones may interact in the regulation of fluid and electrolyte homeostasis.

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18. Radioimmunoassay of AP was performed with a rabbit polyclonal antiserum generated against a cyanogen bromide fragment of high molecular weight AP [C. B. Saper *et al.*, *Science* **227**, 1047 (1985)]. Synthetic APIII was iodinated by the chloramine-T method and purified by high-performance liquid chromatography (HPLC), yielding a specific activity of 200 μCi/μg. This antiserum was 100 percent cross-reactive with API and APII, 10 percent with the high molecular weight precursor atriopeptigen (containing 92 carboxyl terminal amino acids), and did not cross-react with biologically inactive synthetic peptides: residues 13 to 24 of APIII, APIII containing an inversion of Arg-Ile at positions 7 and 8, or arginine-vasopressin. Schwartz *et al.* (*8*) showed a correlation between the plasma immunoreactivity, HPLC migration, biological activity, and sequences of the AP's.

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Ser-Leu-Arg-Arg–Atriopeptin III: The Major Circulating Form of Atrial Peptide

Abstract. *Vasopressin induces a concentration-dependent increase in atriopeptin immunoreactivity in plasma. Rat plasma, rat atrial extract, and synthetic atriopeptin III (APIII) produced parallel displacement curves of iodine-125–labeled APIII binding to specific antiserum. Fractionation of plasma atriopeptin immunoreactivity by reverse-phase high-performance liquid chromatography showed that the major portion consists of two species of low molecular weight peptides in a ratio of 10 to 1. Both peaks exhibited potent vasorelaxant activity, suggesting the presence of the carboxyl terminal Phe-Arg sequence of atriopeptin in each species. Sequence determination of the purified peptides indicated that the major peptide is Ser-Leu-Arg-Arg–APIII and the minor peptide APIII. It appears that the former is the major species of atrial peptide in the rat circulation and that it is the product of selective cleavage of the high molecular weight precursor.*

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Several related low molecular weight peptides with potent natriuretic, diuretic, and vascular smooth muscle relaxant activity have been isolated from mammalian atrial myocytes and their sequences determined (*1–3*). All these atriopeptins (AP's) contain the same core sequence of 17 amino acids within a cystine disulfide bridge, but differ in the lengths of their amino and carboxyl termini. The vasorelaxant property of the AP's requires the presence of the Phe-Arg sequence in the carboxyl terminus (*1, 4, 5*). Variations in the peptide sequence probably result from proteolysis during extraction or processing within the atrial granules.

To establish the endocrine nature of AP's, both stimulated release and structural identification of the circulating form must be demonstrated. Circulating AP's have been detected by radioimmunoassay, suggesting that they have an endocrine role in fluid and electrolyte homeostasis (*6–9*). These studies have shown that (i) atrial extracts, plasma,