Dopaminergic Mediation of the Diuretic and Natriuretic Action of Centrally Administered Rat Atrial Natriuretic Factor (99-126) (42824)

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Abstract. **Intracerebroventricular administration of either rat atrial natriuretic factor (99-126) or dopamine to conscious male hydrated rats resulted in an increase in urinaryvolume and sodium excretion. This activity was prevented, in both cases, by nonselective dopamine antagonist haloperidol (2.5 or 1.25 mg/kg sc, 18 and 2 hr before intracerebroventricular administration of atrial natriuretic factor). Our findings suggest that atrial natriuretic factor exerts its centrally mediated effects on sodium and water metabolism, at least in part, via a dopaminergic mechanism.** [P.S.E.B.M. 1989, Vol 190]

Cardiac atrial myocytes synthesize and store several peptides, including the atrial natriuretic factor (ANF), which have potent diuretic, natriuretic, and vasorelaxant effects (1, 2). Increasing evidence supports the proposition that ANF may play an active role in the regulation of water metabolism and cardiovascular function (1, 2). In addition to the peripheral effects, some actions of ANF could be centrally mediated. In fact, using immunohistochemical techniques, ANF-containing neurons and nerve fibers were identified in rat brain, with the highest concentration of ANF immunoreactivity being found in the hypothalamus and septum (3, 4). In addition, binding sites for ANF have been localized in specific rat brain areas such as the subfornical organ and the anteroventral third ventricular area (AV3V) (5, 6). These observations suggest the possibility that there are central ANF mechanisms that may play a role in the homeostasis of body fluids and electrolytes. Indeed, it has been demonstrated that central administration of ANF has a significant effect on salt appetite (7), water intake (8), vasopressin secretion (9), and urinary volume and sodium excretion (10). The mechanism of rat atrial natriuretic factor-intracerebroventricular (rANF-IVT)-induced diuresis and natriuresis is unknown.

Considerable evidence has accumulated in support of a role for dopamine (DA) as a neurotransmitter in the central nervous system (11). In addition to the well-known ability of DA to inhibit release of prolactin (PRL) (12), it has been suggested that dopaminergic neurons participate in the regulation of blood pressure (13) and thirst (14). Furthermore, although the findings on the action of central DA are controversial (15, 16) they do not discard the possibility that dopaminergic neurons participate in the regulation of vasopressin release (15). The interaction of ANF with brain dopaminergic systems has been demonstrated (17). In addition, third intracerebroventricular injection of ANF inhibits prolactin secretion in a dose-dependent manner (18). Since DA is inhibitory to PRL secretion, the reduction of this hormone levels after IVT-ANF injection could be mediated by this neurotransmitter (19), suggesting a possible role of brain dopaminergic system in the central action of ANF. Thus, we were prompted to evaluate the possible involvement of brain dopaminergic system in the diuresis and natriuresis produced by centrally administered ANF.

Materials and Methods

Adult male Sprague-Dawley rats (230–300 g) were housed under controlled conditions of temperature and photoperiod (lights on from 0600 to 1800 hr) and were provided with free access to laboratory chow and water. A cannula (20) was implanted in the left lateral cerebroventricle, 1 mm caudal to the coronal suture and 1.5 mm lateral to the midsagittal suture, with the aid of a stereotaxic instrument and under pentobarbital anesthesia (40 mg/kg ip). The cannula was secured to the skull with acrylic cement. A minimum of 2 days

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was allowed for recovery. Single IVT injections were made with a Hamilton syringe fitted with a stop to prevent needle penetration past the cannula tip.

Groups of rats received a subcutaneous dose of haloperidol (H) (2.5 and 1.25 mg/kg, $n = 30$) or vehicle (V) (0.3% tartaric acid, $n = 24$) at 18 and 2 hr before IVT-rANF, respectively. The rats were weighed and placed in individual metabolic cages. At 0900 hr, half of the rats receiving each pretreatment were injected IVT, as a bolus in 10 sec, with saline solution (S) (5 μl) or dopamine (100 μg/5 μl) and alternatively with saline solution (5 μl) or freshly prepared rANF in saline solution (5 μg/5 μl) (rat atrial peptide, 28 amino acids; Peninsula Laboratories, Inc., Belmont, CA), followed by 20 ml/kg water orally. Urine was collected at 1, 3, and 6 hr; the bladder was emptied at 6 hr by gentle suprapubic massage. Food and water were not available during the experiment. Ventricular cannula placement was confirmed post mortem by examining the distribution of an IVT injection of 5 μl of fast green dye, which was given before sacrificing the animal. Data were used only if the dye was distributed in the lateral third, and fourth ventricles. Urine samples were assayed for sodium and potassium content by flame photometry.

All data are presented as mean ± SEM. Statistical differences between groups were analyzed using two-way analysis of variance (ANOVA) and by the Newman-Keuls-Student range statistic.

Results

Effects of Haloperidol Pretreatment on Urinary Response to Intracerebroventricular Administration of Dopamine. Urine was collected after 1-, 3-, and 6-hr periods of single IVT injection of DA. The urinary response to IVT-DA in haloperidol-pretreated rats is illustrated in Figure 1. Two-way analysis of variance and the Newman-Keuls test revealed that urine volume was increased 3 hr after administration of DA; this increase was inhibited by haloperidol pretreatment ($V - S < V - DA > H - S = H - DA$; $V - S = H - S = H - DA$) ($F(DA) = 9.9$, $F(H) = 16.9$; $P < 0.01$). Evaluation of sodium excretion revealed a significant increase at 3 hr ($P < 0.01$) in DA-treated rats which was prevented by haloperidol pretreatment ($V - S < V - DA > H - S = H - DA$; $V - S = H - DA$). There was a significant interaction between DA and pretreatment ($FI = 9.4$, $P < 0.01$). Potassium excretion and urinary sodium to potassium ratio were unaffected by DA or by haloperidol pretreatment.

Effects of Haloperidol Pretreatment on rANF-Induced Diuresis and Natriuresis. The effect of IVT-rANF on urine response is illustrated in Figure 2. Two-way ANOVA and the Newman-Keuls test revealed that rANF clearly evoked diuresis at 1-, 3-, and 6-hr periods of urine collection. This effect was prevented by haloperidol pretreatment ($F(A) =$ 1 hr: 10.2, 3 hr: 10.4 and 6 hr: 6.7, $P < 0.01$) ($F(H)$ all $= 10.0$) ($V - S < V - A > H - S = H - A$; $H - S = V - S = H - A$). There

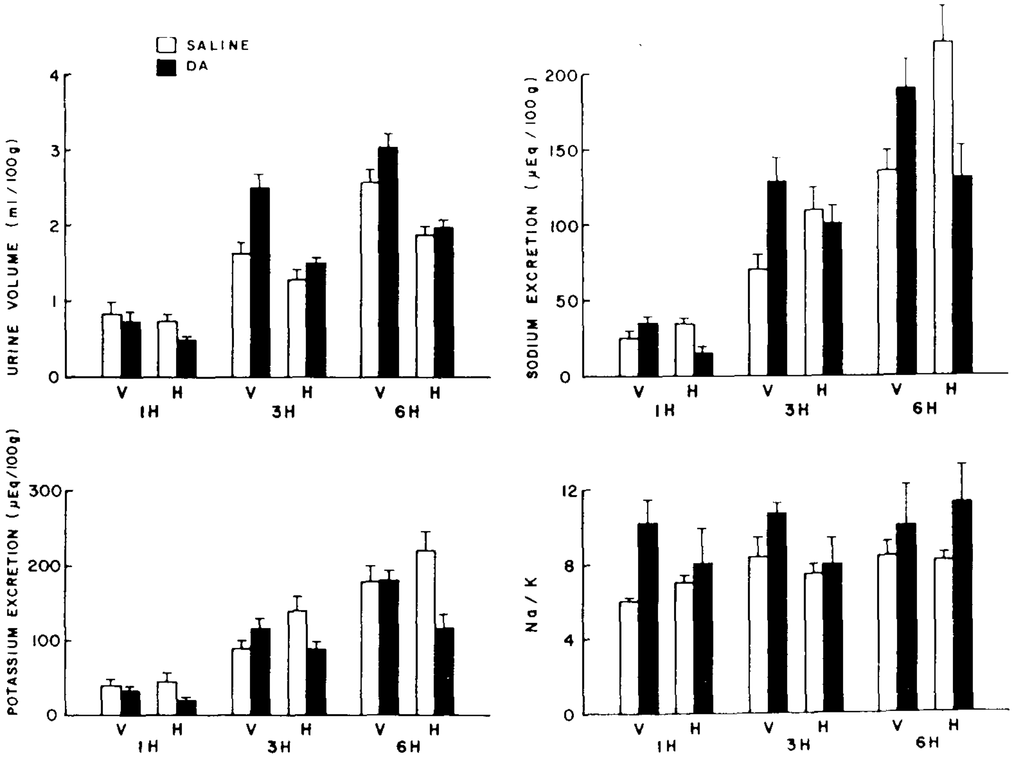

Figure 1. Effects of haloperidol pretreatment on urinary response to IVT-DA. Groups of rats were given subcutaneous injections of V ($n = 24$) or H ($n = 30$) in two doses of 2.5 and 1.25 mg/kg, 18 and 2 hr before IVT-DA (100 μg/5 μl) or saline (5 μl) injection followed by water by mouth. Significant F ratios ($P < 0.01$) from two-way ANOVA were volume and Na^+ effect-DA and haloperidol effect and interaction at 3 hr.

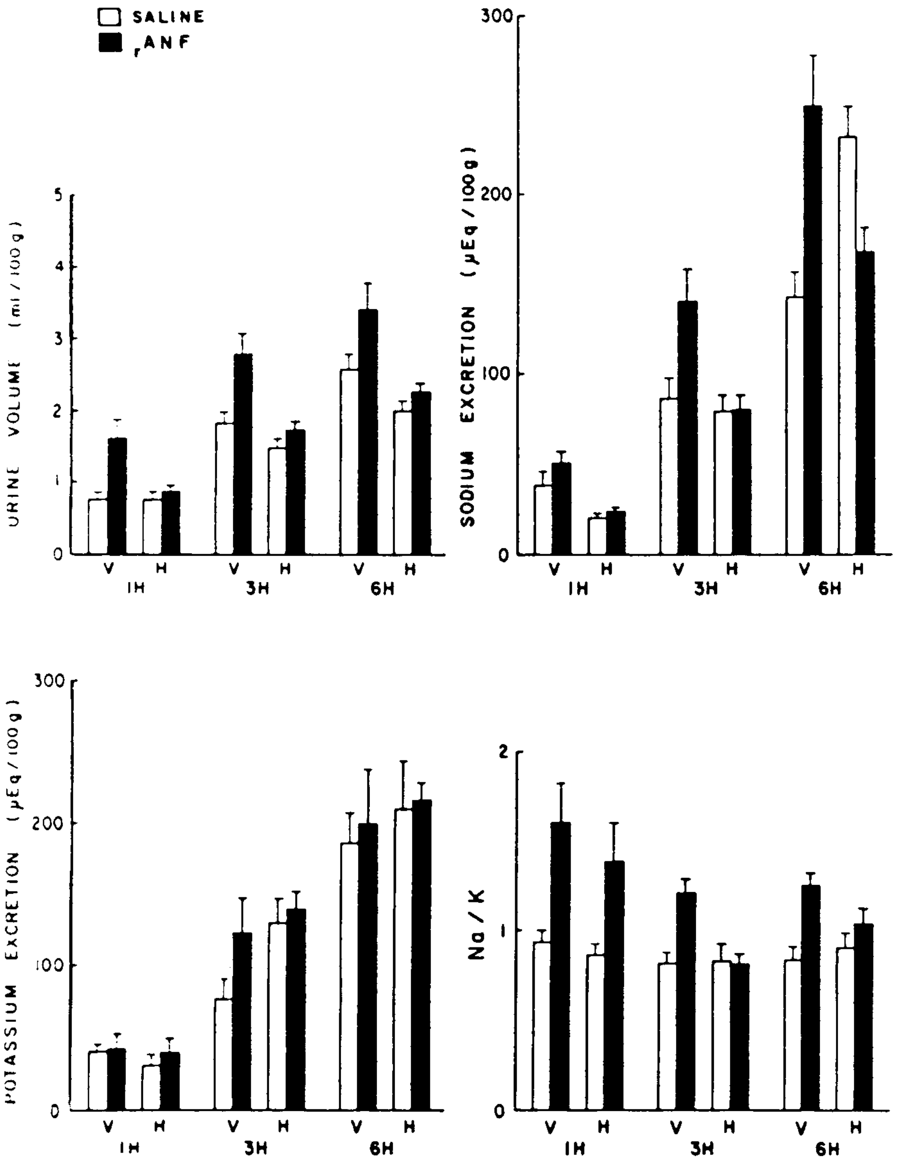

Figure 2. Urinary response to IVT-rANF in rats pretreated with H. Groups of rats were pretreated (sc) with V ($n = 24$) or H ($n = 30$) (see Materials and Methods) and IVT injected with saline (5 μl) or rANF (2.5 μg/5 μl) followed with water by mouth (20 ml/kg). Significant F ratios ($P < 0.01$) from two-way ANOVA were volume- and Na^+-rANF and pretreatment effect at 3 and 6 hr and interaction at 3 and 6 hr. Sodium to potassium ratio-rANF and haloperidol effect at 1, 3, and 6 hr.

was a significant interaction between pretreatment and IVT-rANF injection (F ratios > 5.0, $P < 0.01$). The increase in urinary volume was associated with an enhanced natriuresis at 3- and 6-hr periods of collection. This effect was also prevented by haloperidol pretreatment (F(A) (3 hr) $= 4.5$ and (6 hr) $= 5.0$; $P < 0.05$) ($V - S < V - A > H - S = H - A$; $H - S = V - S = H - A$) (F(H) $= 3.0$ and 6.0, $P < 0.01$). In addition, at 6 hr, haloperidol pretreatment was associated with a significant increase in sodium excretion which was reverted by IVT-rANF injection. There was a significant interaction between treatment and IVT-rANF injection. ANF-induced kaliuresis was not significant; however, an increase associated with haloperidol pretreatment was observed at 3 and 6 hr. Evaluation of the urinary sodium to potassium ratio revealed a significant effect of rANF at 1, 3, and 6 hr that was inhibited by haloperidol pretreatment (F ratios > 3.0, $P < 0.05$) ($V - S < V - A$; $H - S < H - A$ at 1 hr and $V - S < V - A > H - A = H - S$ at 3 and 6 hr).

Discussion

Atrial natriuretic factor when administered as a single IVT injection induces a significant, dose-dependent increase in urine and sodium excretion in conscious rats (10). The exact mechanisms by which central administration of ANF gives rise to natriuresis and diuresis is unknown.

Although several brain areas mediating the central effects of ANF have been characterized (3–6), there is not enough information on the transmitter system(s) involved and the modulation of inhibitory or excitatory factors upon these circuits. Several lines of evidence imply that ANF may exert its effect by an action on adrenergic neurons. It has been demonstrated that chronic ANF infusion suppressed the compensatory increase in norepinephrine excretion secondary to adrenalectomy (21) and the increased sympathetic tone in one-kidney, one-clip renovascular hypertension (22). This evidence indicates an inhibitory modulation of sympathetic outflow by ANF, an action that could be, at least partially, mediated through the CNS. The reduced adrenergic neural tone induced by ANF might alter systemic hemodynamics, which may secondarily influence renal function and sodium excretion. In addition, a reduction in renal adrenergic neural tone may alter renal sodium excretion either indirectly by influencing renal hemodynamics or directly by influencing renal epithelial cell sodium transport, with a resultant natriuresis. In experiments done in our laboratories (23), we demonstrated that chemical sympathectomy did not alter the natriuresis and reduced, but did not prevent, the increase of urine volume induced by IVT-ANF, suggesting that the CNS mechanism of diuresis and natriuresis is independent of the neural tone that the adrenergic system exerts on sodium and water reabsorption.

Intracerebroventricular injection of ANF has been reported to decrease dopamine and its metabolite content in the septum and hypothalamus (17). The changes in hypothalamus DA levels induced by IVT-rANF suggest that the peptide may indeed affect dopaminergic neurones. Furthermore, the suppression of PRL levels after central (IVT), but not peripheral (iv), injection of ANF (18) suggests a central action of the peptide, perhaps at the mediobasal hypothalamus. ANF may stimulate tuberoinfundibular DA neurons to increase the release of DA from terminals in the median eminence (19). DA released from these terminals enters the portal capillaries and is carried by portal veins to the anterior pituitary where it exerts a tonic inhibitory action on PRL release. It has been shown that PRL contributes to the regulation of fluid and electrolyte metabolism in mammals and acts synergistically with angiotensin II to cause drinking and fluid retention (24–27). In addition, ANF may stimulate the dopaminergic tuberohypophyseal system, which originates in the rostral part of the arcuate nucleus and periventricular nucleus of the mediobasal hypothalamus and projects to the neurointermediate lobe of the pituitary (28–30). In the posterior lobe, DA has been postulated to function as either a neurotransmitter, a neurohormone, or both (31) and has been involved in the regulation of water balance (16) and oxytocin secretion (31). In fact, inhibitory effects of ANF on arginine vasopressin secretion have been reported, *in vivo* and *in vitro*, in rats (9, 32–34). In conscious sheep (35), IVT-ANF-induced decrease in arginine vasopressin plasma concentration is associated with an increase in urinary-free water excretion, adding evidence to a centrally mediated renal effect of the peptide.

In the present study we demonstrated that in addition to the increased volume and sodium excretion induced by ANF-IVT, central administration of DA induced the same renal effects, although with a different time course. The dopamine receptor antagonist haloperidol per se, in the dose used in this study, produced an apparent, but not significant reduction of urine volume that was associated with a significant increase in baseline sodium excretion. This apparent volume reduction could represent a direct renal effect of haloperidol, i.e., inhibition of a dopaminergic tone on the urine excretion. Indeed, it has been shown that intravenous injection of 50 μg of haloperidol largely abolished the diuretic and natriuretic response to dopamine and to ANF (36). The mechanism of the natriuretic action of haloperidol is unclear. It is known that haloperidol administered 16 hr before sacrifice induces a delayed increase in the rate of dopamine synthesis and turnover in the terminal region of tuberoinfundibular neurons (i.e., median eminence) (37); this could account for the central-mediated natriuretic effect of haloperidol. In addition, haloperidol administered subcutaneously (present results) or intracerebroventricularly (unpublished results) inhibited the diuretic and natriuretic response to IVT administration of both rANF and DA, suggesting a possible involvement of brain dopaminergic system in the centrally mediated action of ANF on urine and sodium excretions. The close anatomical relationship between brain ANF and dopaminergic systems (3, 4, 12, 28–30, 38), found to be jointly involved in the central control of blood pressure and fluid balance (1, 2, 14, 38), support the possibility of such interaction. In effect, the concept of some interaction between ANF and the dopamine system is supported by the fact that several actions of ANF are similar to those produced by the activation of the dopamine system, including effects on blood pressure and plasma levels of PRL (12, 14, 18). Furthermore, both substances, when administered IVT, enhance renal sodium excretion and urine volume (10, 39, 40; present results) and both inhibit the release of PRL (12, 18) and vasopressin (9, 16).

Our data suggest a contribution of brain dopaminergic systems to the expression of central ANF-mediated diuresis and natriuresis. It seems possible that ANF is able to modify the activity of the tuberoinfundibular and/or tuberohypophyseal DA neurons and in this way participate in the regulation of homeostasis of fluid and electrolite.

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