Dopamine Inhibits Vasopressin Action in the Rat Inner Medullary Collecting Duct via α₂-Adrenoceptors

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ABSTRACT

We compared the effects of dopamine and norepinephrine on vasopressin (AVP)-stimulated increases in osmotic water permeability (Pf) and cAMP accumulation in the rat inner medullary collecting duct (IMCD). Both dopamine and norepinephrine inhibited AVP-induced Pf and cAMP accumulation in a concentration-dependent manner; however, norepinephrine was approximately 100-fold more potent than dopamine. The effects of dopamine on Pf were antagonized by the selective α₂-adrenoceptor antagonist, rauwolscine (10 nM–1 μM), dopamine D₄ receptor antagonist, clozapine (10 μM), and norepinephrine-induced decreases in AVP-stimulated Pf were antagonized by the selective α₂-adrenoceptor antagonists, rauwolscine, idazoxan, and yohimbine, but not by the dopamine receptor antagonists, spiperone, SCH-23390, or raclopride. Clozapine (1–10 μM) inhibited the effects of both dopamine and norepinephrine on AVP-stimulated cAMP levels. We conclude that the inhibitory effects of dopamine on AVP-induced Pf and cAMP accumulation in the rat IMCD are mediated via α₂-adrenoceptors.

It is generally accepted that dopamine is an important regulator of renal function (Lee, 1993). When administered to humans and other species, dopamine has marked natriuretic and diuretic effects (Jose et al., 1992; Lee, 1993). Although the hemodynamic actions of dopamine (increased renal blood flow and glomerular filtration rate) undoubtedly contribute to these effects on renal function, direct tubular actions of dopamine are also likely to be involved (Jose et al., 1992; Holtback et al., 2000). The proximal tubule and inner medullary collecting duct (IMCD) cells in culture synthesize dopamine (Huo et al., 1991; Jose et al., 1992), and the natriuretic effects of this catecholamine appear to be part of an intrarenal paracrine or autocrine system (Siragy et al., 1989; Jose et al., 1992; Holtback et al., 2000). Dopamine receptors have been identified in various segments of the nephron. Results from pharmacological, ligand binding, and reverse transcriptase-polymerase chain reaction studies have provided evidence for D₁, D₂, D₃, and D₄ receptors associated with various tubule segments (Meister et al., 1991; Takemoto et al., 1991; Gao et al., 1994; O’Connell et al., 1995, 1998; Sun et al., 1998). Functional effects attributed to dopamine include inhibition of Na⁺/K⁺-ATPase in a number of tubule segments (Bertorello and Katz, 1993), inhibition of fluid absorption, Na⁺/H⁺ exchange and phosphate transport in the proximal tubule (Kaneda and Bello-Reuss, 1983; Felder et al., 1990; Baum and Quigley, 1998), and inhibition of vasopressin-stimulated osmotic water permeability (Pf) and Na⁺ transport in the cortical collecting tubule (Muto et al., 1985; Sun and Schafer, 1996). Although most of the proximal tubule effects of dopamine appear to be due to activation of D₁ receptors (Felder et al., 1990; Baum and Quigley, 1998; Holtback et al., 2000), a series of recent studies (Sun and Schafer, 1996; Li and Schafer, 1998; Sun et al., 1998) suggests that dopamine’s effects on vasopressin-dependent water permeability and Na⁺ transport in the rat cortical collecting tubule are mediated by a D₄-like receptor. This was based on observations that D₄ receptor mRNA and protein are expressed throughout the collecting duct system (Sun et al., 1998), and agonists and antagonists of D₁, D₂, and D₄ receptors failed to mimic or attenuate the inhibitory effects of dopamine on AVP-dependent water, Na⁺ transport (Sun and Schafer, 1996), and AVP-stimulated cAMP accumulation (Li and Schafer, 1998). However, clozapine, an “atypical” neuroleptic with D₄ antagonist activity (Van Tol et al., 1991), did attenuate the dopamine-mediated effects on vasopressin action (Sun and Schafer, 1996; Li and Schafer, 1998). In the present study, the effects of dopamine were examined on AVP-dependent water permeability and cAMP accumulation in the rat IMCD to determine whether a similar system is operable in this segment of the nephron. Since high concentrations of dopamine can activate α₂-adrenoceptors (Phillips, 1980), which are known to inhibit AVP action in the IMCD (Ed-
wards and Gellai, 1988), the effects of norepinephrine were studied in parallel. Contrary to expectations, the results of the present study suggest that the effects of dopamine on AVP action in the IMCD can be attributable to activation of $\alpha_2$-adrenoceptors.

**Materials and Methods**

**Perfused Tubules.** Tubules were perfused as previously described (Edwards and Spielman, 1994). Kidneys were removed from anesthetized (pentobarbital, 50 mg/kg i.p.) male Sprague-Dawley rats (250–300 g, Charles River Laboratories, Inc., Wilmington, MA) that had free access to standard laboratory chow and water. Corticomedullary slices were placed in chilled bath solution (see below) containing 0.1% bovine serum albumin to facilitate dissection. IMCDs were dissected from the lower two-thirds of the inner medulla, transferred to a temperature-controlled chamber, and mounted on micropipettes. Perfused tubule length averaged 786 ± 30 μm ($n = 33$). Tubules were initially perfused and bathed with a hypertonic solution consisting of 210 mM NaCl, 5 mM KCl, 1.5 mM CaCl$_2$, 1.2 mM MgSO$_4$, 2.3 mM Na$_2$HPO$_4$, 8 mM glucose, 5 mM alanine, and 10 mM HEPES. The pH and osmolality were adjusted to 7.4 with NaOH and 450 mosmol/kg H$_2$O with NaCl, respectively. The temperature of the bath was gradually increased and maintained at 37°C. Prewarmed bath solution was continuously pumped through the chamber at 0.5 ml/min. Thirty minutes after the chamber had reached 37°C, the perfusate was changed to an isotonic solution (300 mosmol/kg H$_2$O) that was identical with the bath except that it contained less NaCl (135 mM) and dialyzed [3H]inulin, which served as a volume marker. Timed collections of perfusate were made using a constant volume pipette, and Pf (μm/s) was calculated according to Al-Zahid et al. (1977). Tubules were perfused at rates of 20 to 30 nl/min to prevent osmotic equilibrium between the perfusate and bath and did not differ between control and experimental periods.

Most experiments consisted of three collection periods: a control period, an experimental period, and an additional control period at the end of the experiment. Approximately 20 min after changing to an isotonic perfusate, the bath was changed to one containing a near-maximal concentration of AVP, 10 pM (Nadler et al., 1992), to which the tubule was exposed for the remainder of the experiment. Thirty to 40 min following the addition of AVP, three to four collections were made to determine AVP-stimulated Pf (control period). Test compounds (e.g., dopamine) were then added to the AVP-containing bath, and 15 min later, three to four collections were made (experimental period). Test compounds were then removed from the bath, and following a 15-min equilibration period, an additional three to four collections were made in the presence of AVP alone. An identical protocol was used when the cAMP analog, 8-p-chlorophenylthio-cAMP (CPT-cAMP), was used in place of AVP. Concentration-response experiments were performed in a similar manner except that each tubule was exposed to sequentially higher concentrations of the compounds, separated by 15-min equilibration periods. For each experiment, a Pf value for a given period was determined by averaging the values obtained from three to four collections. Results are expressed as Pf in absolute terms or as a percentage of control values. Statistical analysis was performed with Student's t test for paired comparisons or by analysis of variance followed by Tukey's test for multiple comparisons. Statistical analysis and curve fitting were performed using GraphPad Prizm software (GraphPad Software, San Diego, CA).

**Reagents.** [3H]Inulin and cAMP radioimmunoassay kits were obtained from PerkinElmer Life Science Products (Boston, MA). AVP, dopamine HCl, norepinephrine bitartrate, and clozapine were obtained from Sigma (St. Louis, MO). Rauwolscine, yohimbine, idazoxan, SCH-23390, raclopride, and spiperone were obtained from Sigma/RBI (Natick, MA). Dopamine and norepinephrine stock solutions were made up in 0.1% acetic acid and protected from light. Concentrated stock solutions (1 mM) of rauwolscine, yohimbine, idazoxan, SCH-23390, spiperone, and raclopride were made up in water. Clozapine (20 mM) was made up in 0.1 N HCl and diluted with buffer. All control and experimental solutions also contained the appropriate vehicle.

**Results**

Dopamine produced a rapid and reversible decrease in AVP-induced Pf (Fig. 1). In the presence of 10 pM AVP, Pf averaged 947 ± 89 μm/s. Addition of 10 μM dopamine to the bath decreased Pf to 141 ± 27 μm/s ($p < 0.001$). Upon removal of dopamine, Pf increased to 890 ± 99 μm/s, a value not different from the initial AVP period. In contrast to its effect on AVP-induced Pf, dopamine (10 μM) had no effect on AVP-induced Pf. Dopamine (10 μM) had no effect on Pf.
Pf stimulated by the cAMP analog, CPT-cAMP (Fig. 1). Pf in the presence of 100 μM CPT-cAMP was 690 ± 32 μm/s and did not change when 10 μM dopamine was added to the bath (659 ± 50 μm/s). Figure 2 shows the concentration-dependent effects of dopamine and, for comparison, norepinephrine on AVP-stimulated Pf. Both catecholamines produced a concentration-dependent inhibition of vasopressin-stimulated Pf. Although both compounds inhibited vasopressin action to the same extent, norepinephrine was significantly (p < 0.03) more potent than dopamine. The concentration of norepinephrine needed to inhibit vasopressin-induced Pf by 50% (IC50) was 16.6 ± 4.0 nM compared with 1.9 ± 0.7 μM for dopamine. In contrast to their effects on AVP-stimulated Pf, dopamine and norepinephrine had no effects on basal Pf, which was measured in the absence of AVP. Thus, Pf was 107.9 ± 8.8 and 100.3 ± 11.6 μm/s in the absence and presence of dopamine (10 μM, n = 6) and 112.4 ± 16.3 and 120.6 ± 10.3 μm/s in the absence and presence of norepinephrine (10 μM, n = 5).

Since high concentrations of dopamine can activate α2-adrenoceptors, which are known to inhibit vasopressin action (Edwards and Geladi, 1988; Chen et al., 1991), the effects of the α2-adrenoceptor antagonist, rauwolscine, on the inhibitory effect of dopamine was examined. In these experiments, 10 μM dopamine decreased AVP-stimulated Pf from 966 ± 38 to 126 ± 29 μm/s (Fig. 3). In the presence of dopamine, the addition of increasing concentrations of rauwolscine led to a progressive attenuation of the inhibitory effect of dopamine. Pf increased to 242 ± 42, 608 ± 45, and 842 ± 83 μm/s in the presence of 10 nM, 100 nM, and 1 μM rauwolscine, respectively. Pf in the presence of 1 μM rauwolscine was not significantly different from AVP alone. Following removal of rauwolscine, Pf decreased to 234 ± 18 μm/s, a value not different from the initial dopamine period. Clozapine, an atypical neuroleptic with some selectivity for the D4 receptor (Van Tol et al., 1991), has previously been shown to inhibit the effects of dopamine on AVP-stimulated Pf,Na+ transport, and cAMP levels in the rat cortical collecting tubule (Sun and Schafer, 1996; Li and Schafer, 1998). Consistent with that observation, clozapine (10 μM) partially attenuated the inhibitory effect of dopamine (10 μM) on AVP-dependent Pf (Fig. 4), however, clozapine also inhibited the effects of norepinephrine (1 μM) on AVP-dependent Pf to the same degree.

In IMCDs stimulated with 1 nM AVP, both dopamine and norepinephrine caused a concentration-dependent inhibition of cAMP accumulation (Fig. 5). The IC50 value for dopamine was 1.3 ± 0.39 μM, whereas norepinephrine was more potent (p < 0.05) with an IC50 value of 9.6 ± 1.4 nM. There was a direct correlation between the decrement in AVP-stimulated cAMP levels and Pf produced by norepinephrine (r = 0.99) and by dopamine (r = 0.97). Furthermore, the difference in potency between norepinephrine and dopamine in producing these effects on cAMP levels (135-fold) and Pf (118-fold) was similar. As was the case for dopamine-induced inhibition of AVP-stimulated Pf (Fig. 4), the α2-adrenoceptor antagonist, rauwolscine, inhibited dopamine effects on AVP-stimulated cAMP accumulation in a concentration-dependent manner (Fig. 6). In the presence of 1 μM rauwolscine, the inhibitory effect of dopamine was totally abolished. To investigate further the receptor involved in dopamine’s action, a number of structurally diverse, selective antagonists of α2-adrenoceptors (rauwolscine, yohimbine, and idazoxan) and dopamine

![Fig. 2. Concentration-dependent inhibition of vasopressin-induced Pf (10 pM) by dopamine (○) and norepinephrine (●). Results are expressed as a percentage of vasopressin-induced Pf, which was 905 ± 93 μm/s for the dopamine series (n = 5) and 836 ± 86 μm/s for the norepinephrine series (n = 4).](image-url)
receptors (SCH-23390, D1; spiperone, D2; and raclopride, D2) were tested for their ability to antagonize dopamine-induced inhibition of AVP-stimulated cAMP accumulation. At a concentration of 10^{-6} M, which is well above the Ki values of the dopamine antagonists for their respective receptors (Gingrich and Caron, 1993), only the 2-adrenoceptor antagonists, rauwolscine, yohimbine, and idazoxan, attenuated dopamine’s action on AVP-induced cAMP accumulation (Fig. 7). Clozapine attenuated the effects of both dopamine and nor-epinephrine on AVP-stimulated cAMP accumulation consistent with its effects on Pf (Fig. 8).

Discussion

Dopamine has a number of effects on water and electrolyte transport in the kidney (Lee, 1993). With respect to the collecting tubule, previous studies have shown that dopamine inhibits AVP-induced increases in Pf in the rabbit cortical collecting tubule (Muto et al., 1985) and the rat cortical collecting tubule (Sun and Schafer, 1996). In the rabbit cortical collecting tubule, the effects of dopamine were inhibited by the nonselective antagonist, metoclopramide (Muto et al., 1985), whereas the effects of dopamine in the rat cortical collecting tubule were antagonized by clozapine, a relatively selective D4 receptor antagonist, but not by D1-, D2-, or D3-selective antagonists (Sun and Schafer, 1996; Li and Schafer, 1998). We undertook the present series of experiments to determine whether dopamine inhibits AVP action in the IMCD, the segment of the nephron responsible for the final elaboration of the urine, and a segment of the collecting duct system in which D4 receptor mRNA has been detected (Sun et al., 1998). Furthermore, since dopamine can activate α2-adrenoceptors (Phillips, 1980), which have well characterized inhibitory effects on AVP action in the rat collecting tubule (Chen et al., 1991; Edwards and Gellai, 1988), we examined the effects of norepinephrine and dopamine in parallel.

In agreement with the studies cited above, we found that dopamine caused a concentration-dependent inhibition of
cloned D2 receptor (Asghari et al., 1994). Thus, if dopamine was acting via the D2 receptor, sipiperone should have demonstrated some antagonistic activity. Our data therefore indicate that dopamine inhibits AVP action in the rat IMCD by activating α2-adrenoceptors. Whether or not a similar situation occurs in the rat cortical collecting tubule is not known, since the effects of α2-adrenoceptor antagonists on dopamine-induced inhibition of AVP action have not been studied (Sun and Schafer, 1996; Li and Schafer, 1998).

Although our results indicate that dopamine acts through α2-adrenoceptors to inhibit AVP action in the IMCD, we do not rule out a possible role for this catecholamine in the regulation of salt and water transport in this nephron segment. Should dopamine concentrations reach high enough levels in the inner medulla from local synthesis (Huo et al., 1991) or from the proximal tubule via the postglomerular circulation, dopamine could modulate AVP action by activating α2-adrenoceptors in the IMCD or alter electrolyte transport by action at D4 receptors or other dopamine receptor subtypes yet to be localized to this nephron segment.

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AVP-induced Pf and cAMP accumulation in the rat IMCD. Also consistent with the rat cortical collecting tubule study (Sun and Schafer, 1996) was our observation that dopamine had no effect on the increase in Pf caused by CTP-cAMP. These data, coupled with the inhibition of AVP-stimulated cAMP accumulation, suggest that dopamine inhibits AVP action at the level of adenylate cyclase. However, unlike the conclusions derived from the rat cortical collecting tubule study (Sun and Schafer, 1996), our results are more consistent with dopamine acting via α2-adrenoceptors to inhibit AVP action in the IMCD. This conclusion is based primarily on the following observations. First, norepinephrine produced identical effects to that of dopamine but was more than 100-fold more potent at inhibiting both AVP-induced increases in Pf and cAMP levels. This is the opposite of what one would expect if the D4 receptor was involved, since norepinephrine is greater than 100-fold less potent than dopamine in activating this receptor (Van Tol, 1998). Second, a number of selective α2-adrenoceptor antagonists, including rauwolscine, yohimbine, and idazoxan, antagonized the effects on AVP-induced Pf and cAMP accumulation in the IMCD by clozapine is probably due to antagonism of the partial attenuation of both dopamine and norepinephrine receptors (spiperone, raclopride) had no effect on dopamine-induced inhibition of AVP-stimulated cAMP levels, thus, ruling out a role for these dopamine receptor subtypes. This latter observation is consistent with the findings of Sun and Schafer (1996) and Li and Schafer (1998) in the rat cortical collecting tubule in which D1, D2, or D3 antagonists had no effect on dopamine-induced inhibition of Pf, sodium transport, or cAMP levels stimulated by AVP.

Of the various dopamine receptor antagonists tested, only clozapine inhibited dopamine’s effect on AVP action in the IMCD. This is also consistent with previous observations in the rat cortical collecting tubule (Sun and Schafer, 1996; Li and Schafer, 1998). However, we also found that clozapine attenuated norepinephrine-induced inhibition of AVP-dependent Pf and cAMP levels. Clozapine is a so-called atypical neuroleptic, which has activity at a number of different receptors, including various subtypes of the dopamine, serotonergic, and adrenergic families (Millan et al., 1998). Although clozapine does show some selectivity for the D3 receptor over other dopamine receptor subtypes (Millan et al., 1998), pertinence to the present study are the findings that the Kᵢ for clozapine at the rat cerebral cortex α2-adrenoceptor (67 nM) (Millan et al., 1998) is similar to that found at the rat cloned D3 receptor (90 nM) (Gazi et al., 2000). Therefore, the partial attenuation of both dopamine and norepinephrine effects on AVP-induced Pf and cAMP accumulation in the IMCD by clozapine is probably due to antagonism of α2-adrenoceptors. Furthermore, sipiperone, which had no effect on dopamine-induced inhibition of AVP-stimulated cAMP levels in this study or in the rat cortical collecting tubule (Li and Schafer, 1998), has a higher affinity for the rat cloned D4 receptor (4 nM) (Gazi et al., 2000) than does clozapine (90 nM). A greater affinity for sipiperone (0.07 nM) than for clozapine (22 nM) has also been observed with the human cloned D2 receptor (Asghari et al., 1994). Thus, if dopamine was acting via the D3 receptor, sipiperone should have demonstrated some antagonistic activity. Our data therefore indicate that dopamine inhibits AVP action in the rat IMCD by activating α2-adrenoceptors. Whether or not a similar situation occurs in the rat cortical collecting tubule is not known, since the effects of α2-adrenoceptor antagonists on dopamine-induced inhibition of AVP action have not been studied (Sun and Schafer, 1996; Li and Schafer, 1998).

Although our results indicate that dopamine acts through α2-adrenoceptors to inhibit AVP action in the IMCD, we do not rule out a possible role for this catecholamine in the regulation of salt and water transport in this nephron segment. Should dopamine concentrations reach high enough levels in the inner medulla from local synthesis (Huo et al., 1991) or from the proximal tubule via the postglomerular circulation, dopamine could modulate AVP action by activating α2-adrenoceptors in the IMCD or alter electrolyte transport by action at D4 receptors or other dopamine receptor subtypes yet to be localized to this nephron segment.


