Inhibition of Water Permeability in the Rat Collecting Duct: Effect of Imidazoline and Alpha-2 Compounds[2] (44396)

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Abstract. **Arginine vasopressin (AVP) increases water permeability in the collecting duct of the nephron *via* activation of adenylyl cyclase. Alpha-2 (α_2) agonists inhibit AVP-stimulated water permeability *via* binding to α_2 adrenoceptors that have been divided into 3 subtypes- α_{2A}, α_{2B}, and α_{2C}. Some biological effects mediated by α_2 agonists result from nonadrenergic imidazoline receptors that exist in the rat kidney. Thus, α_2-inhibition of AVP-stimulated water permeability in the rat collecting duct could be caused by imidazoline receptors. The purpose of this study was to test agonists and antagonists selective for α_2 and imidazoline receptors on AVP-stimulated water permeability in the rat inner medullary collecting duct (IMCD). Some experiments were conducted where water permeability was stimulated by a nonhydrolyzable analog of adenosine 3′,5′-cyclic monophosphate (cAMP). Agonists included dexmedetomidine, clonidine, oxymetazoline, agmatine and rilmenidine. The latter two are selective imidazoline agonists. Antagonists included yohimbine, RX821002, atipamezole, prazosin, WB4101, idazoxan, and BU239. Prazosin and WB4101 demonstrate selectivity for the α_{2B} and α_{2C} subtypes, respectively, and oxymetazoline and RX821002 are selective for the α_{2A} subtype. BU239 is selective for imidazoline receptors. Wistar rat terminal IMCDs were isolated and perfused to determine the osmotic water permeability coefficient (P_f). All agonists except agmatine inhibited AVP-stimulated P_f. Inhibition by rilmenidine indicated a different mechanism of action from other agonists. Dose-response data show dexmedetomidine to be the most potent inhibitor. Oxymetazoline and clonidine inhibited cAMP-stimulated P_f indicating that the mechanism involves postcAMP cellular events. It was reported previously that dexmedetomidine inhibits cAMP-stimulated P_f (1). All antagonists except prazosin and WB4101 reversed α_2-inhibition of AVP-stimulated P_f. BU239 was effective at 1 μ*M* but not at 100 n*M*. Results suggest that α_{2A} adrenoceptors modulate water permeability in the IMCD. The involvement of imidazoline receptors is inconclusive.**

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[2] Presented in part at the Experimental Biology '97 meeting, Rouch AJ, Kudo L, and Hebert C. Idazoxan partially reverses alpha-2 inhibition of osmotic water permeability in rat inner medullary collecting duct. FASEB J 11:A459, 1997. Hebert C, Rouch A, and Kudo L. Oxymetazoline inhibits osmotic water permeability in the rat cortical collecting duct and inner medullary collecting duct. FASEB J 11:A22, 1997.

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Alpha-2 (α_2) agonists inhibit arginine vasopressin (AVP)-stimulated water permeability in the rat cortical collecting duct (CCD) and inner medullary collecting duct (IMCD). Some of these agonists include clonidine (2), dexmedetomidine (1), and epinephrine (3). The first two are selective α_2 agonists, and the third is a nonselective adrenergic agonist that binds to α_1, α_2, and β-adrenoceptors. Epinephrine-induced inhibition is reversed by the α_2 antagonist yohimbine but not by the α_1 antagonist corynanthine (3).

This inhibitory mechanism is attributed to α_2-induced reduction of adenylyl cyclase activity *via* activation of an inhibitory G protein. Consequently, the α_2 agonist reduces

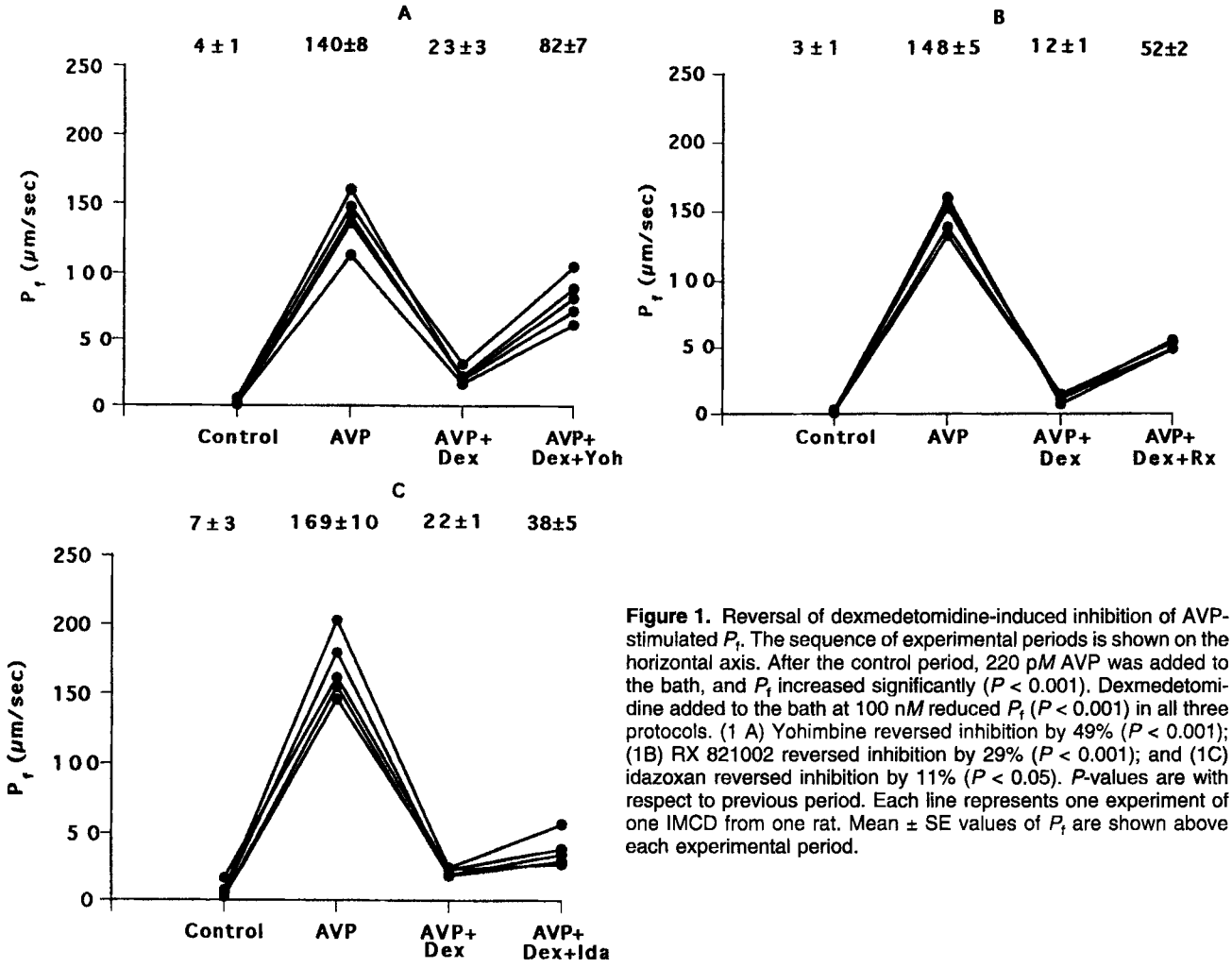

Figure 1. Reversal of dexmedetomidine-induced inhibition of AVP-stimulated P_f. The sequence of experimental periods is shown on the horizontal axis. After the control period, 220 p*M* AVP was added to the bath, and P_f increased significantly ($P < 0.001$). Dexmedetomidine added to the bath at 100 n*M* reduced P_f ($P < 0.001$) in all three protocols. (1 A) Yohimbine reversed inhibition by 49% ($P < 0.001$); (1B) RX 821002 reversed inhibition by 29% ($P < 0.001$); and (1C) idazoxan reversed inhibition by 11% ($P < 0.05$). *P*-values are with respect to previous period. Each line represents one experiment of one IMCD from one rat. Mean ± SE values of P_f are shown above each experimental period.

cellular levels of adenosine 3′,5′-cyclic monophosphate (cAMP), the second messenger that leads to increased water permeability in the IMCD (4). However, we reported that α_2 agonists inhibit water permeability subsequent to being stimulated by nonhydrolyzable analogs of cAMP (1, 5). This evidence suggests that other cellular messengers and/or two or more receptor subtypes have biological roles in this mechanism.

Three α_2-adrenoceptor subtypes have been identified—α_{2A}, α_{2B}, α_{2C}—and at least two and perhaps all three exist in the rat kidney (6, 7). Moreover, some receptor-mediated effects previously thought to have been induced *via* α_2-adrenoceptors actually occurred *via* nonadrenergic imidazoline receptors (8, 9). Only a few α_2 agonists and antagonists have been tested on water permeability in isolated collecting duct nephron segments, and no specific imidazoline-selective agents have been tested.

The purpose of this study was to test the ability of specific α_2 and imidazoline agonists to inhibit AVP- and cAMP-stimulated water permeability in the rat IMCD. These agonists included dexmedetomidine, clonidine, oxymetazoline, agmatine, and rilmenidine. Antagonists were tested to determine if they would reverse inhibition. These included atipamezole, yohimbine, RX821002, idazoxan, prazosin, WB4101, and BU239. Results strengthened evidence indicating that α_2-inhibition of water permeability in the IMCD is a more complex mechanism than just α_2-induced inhibition of adenylyl cyclase. They also suggested that the α_{2A}-adrenoceptor subtype is involved in the mechanism. Rilmenidine induces inhibition *via* an apparent different mechanism.

Materials and Methods

IMCD segments were isolated and perfused by conventional techniques previously described (10, 11). Wistar rats were sacrificed by decapitation, and the kidneys were rapidly removed and cut into small slices that were placed in chilled dissection solution of the same composition as the bathing solution or bath described below. IMCD segments were dissected and isolated from the terminal two-thirds of the inner medulla (i.e., the terminal IMCD) (12, 13).

After isolation, the IMCD was transferred to a perfusion chamber on the stage of an inverted microscope and mounted on concentric pipettes that suspended the tubule in the bath. One end of the tubule was drawn by suction into the tip of one of the outer pipettes. The tip of the inner

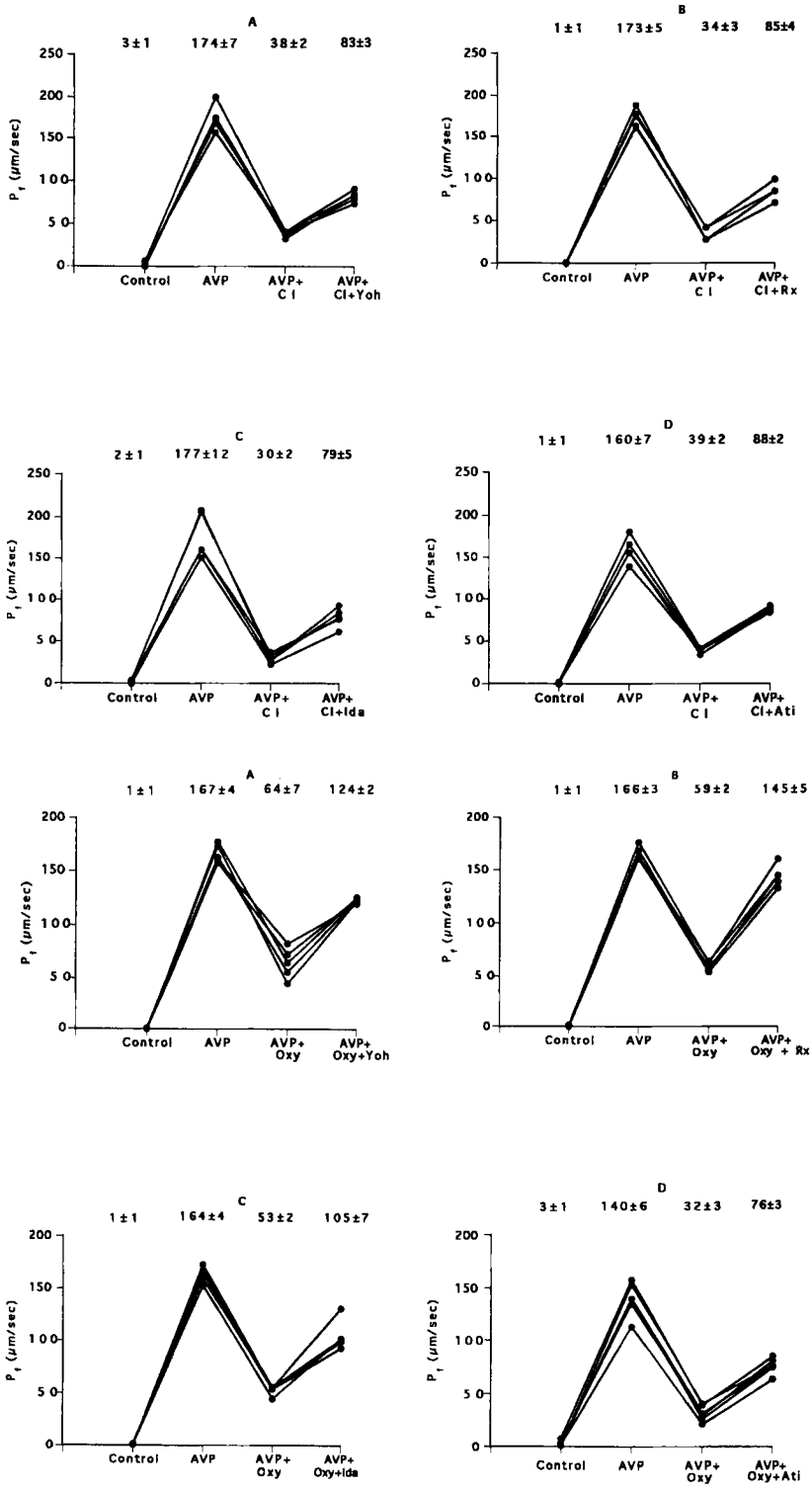

Figure 2. Reversal of clonidine-induced inhibition of AVP-stimulated P_f. After the control period, 220 p*M* AVP was added to the bath and P_f increased significantly ($P < 0.001$). Clonidine added to the bath at 100 n*M* reduced P_f ($P < 0.001$) in all four protocols. (2 A) Yohimbine reversed inhibition by 33% ($P < 0.001$); (2B) RX 821002 reversed inhibition by 39% ($P < 0.001$); (2C) idazoxan reversed inhibition by 33% ($P < 0.001$); and (2D) atipamezole reversed inhibition by 40%. (For format, see legend Fig. 1)

Figure 3. Reversal of oxymetazoline-induced inhibition of AVP-stimulated P_f. After the control period, 220 p*M* AVP was added to the bath, and P_f increased significantly ($P < 0.001$). Oxymetazoline added to the bath at 100 n*M* reduced P_f ($P < 0.001$) in all four protocols. (3A) Yohimbine reversed inhibition by 58% ($P < 0.001$); (3B) RX 821002 reversed inhibition by 80% ($P < 0.001$); (3C) idazoxan reversed inhibition by 47% ($P < 0.001$); and (3D) atipamezole reversed inhibition by 39%. (For format, see legend Fig. 1)

pipette containing the luminal perfusion solution, or perfusate, was advanced into the lumen of the tubule, and perfusion was initiated *via* air pressure.

The opposite end of the tubule was held in the tip of another glass micropipette where the perfusate accumulated. The tip of this pipette was coated with a viscous silicone liquid (Sylgard 184, Dow Corning, Midland, MI) to isolate the perfusate from the bath. Samples of collected perfusate were taken periodically during the experiment with a constant-volume or volumetric pipette. The bath composition was as follows (in m*M*): 115 NaCl, 25 $NaHCO_3$, 10 sodium acetate, 5 KCl, 1.0 $CaCl_2$, 1.2 $MgSO_4$, 1.2 NaH_2PO_4, 5.5 glucose, and the pH = 7.4. The solution was continuously bubbled with 95% O_2/5% CO_2 gas. All experiments were conducted at 37°C.

The osmotic water permeability coefficient (P_f) was

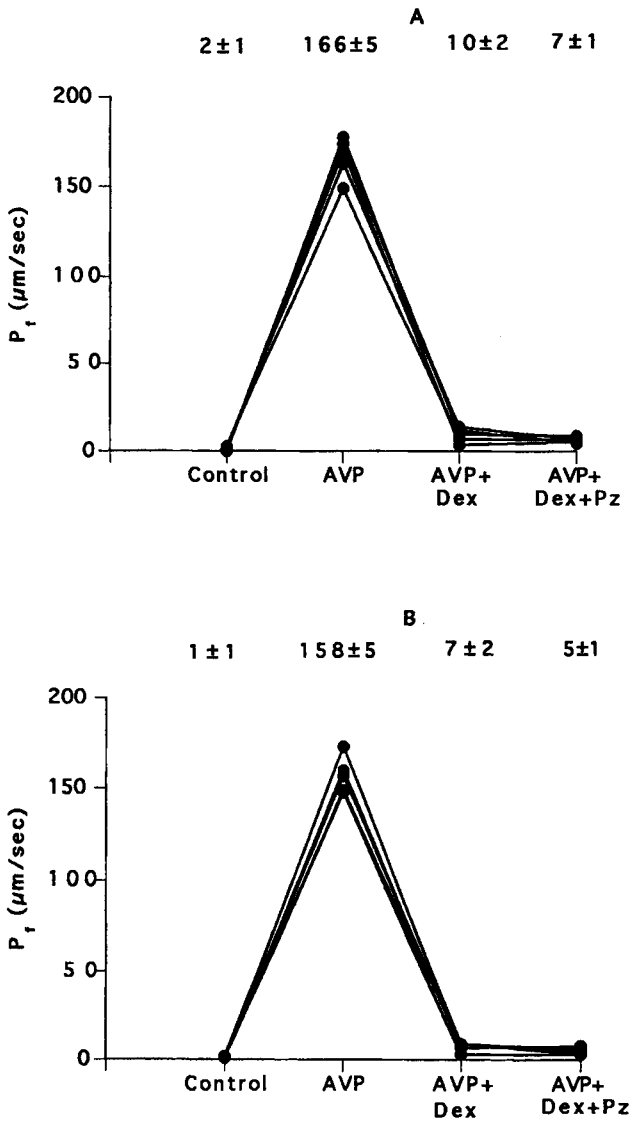

Figure 4. Prazosin failed to reverse dexmedetomidine-induced inhibition of AVP-stimulated P_f. After the control period, 220 p*M* AVP was added to the bath, and P_f increased significantly ($P < 0.001$). Dexmedetomidine added to the bath at 100 n*M* reduced P_f ($P < 0.001$) in both protocols. Prazosin (Pz) at 100 n*M* (4 A) or 1 μ*M* (4B) failed to reverse inhibition. (For format, see legend Fig. 1)

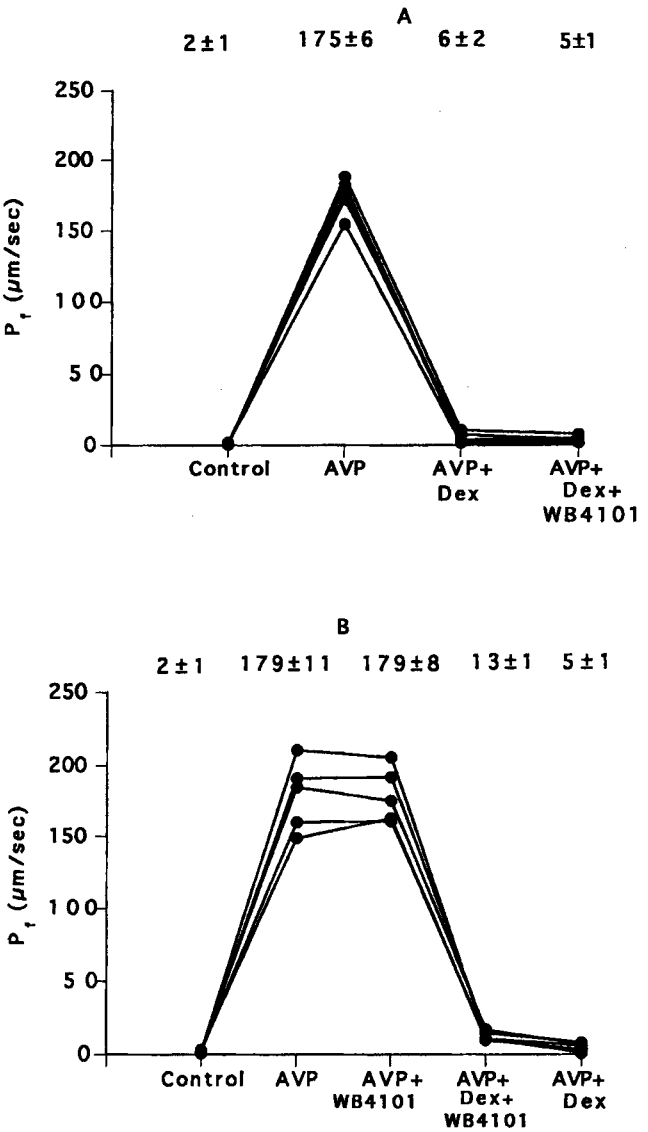

Figure 5. WB4101 failed to reverse dexmedetomidine-induced inhibition of AVP-stimulated P_f. AVP added to the bath at 220 p*M* increased P_f ($P < 0.001$). In 5A, dexmedetomidine at 100 n*M* decreased P_f ($P < 0.001$) and WB4101 at 1 μ*M* failed to reverse inhibition. In 5B, WB4101 at 1 μ*M* did not affect AVP-stimulated P_f and did not prevent dexmedetomidine from reducing P_f. (For format, see legend Fig. 1)

determined by measuring net fluid flux (J_v) in the presence of a lumen-to-bath osmotic gradient (80–90 to 295–300 mOsm/kg H_2O). The perfusate was made hypotonic to the bath by reducing the NaCl concentration to 50 m*M*. ^{14}C-inulin in the perfusate served as the volume marker, and rapid luminal perfusion rates of 20–30 nl/min were used to avoid osmotic equilibration. Perfusion rate (V_i) was calculated as $V_i = V_o(In_o/In_i)$, where In_i and In_o are the inulin activities (in cpm/nl) in the initial perfusate and collected fluid, respectively. The collection rate, V_o, was determined directly by measuring the time required to fill the volumetric pipette. J_v was calculated as: $J_v = (V_i - V_o)/L$ where L is the tubule length measured with an eyepiece micrometer. P_f was calculated using standard equations (14).

Experiments contained four or five periods where three timed-fluid samples were collected in each period. P_f was calculated for each sample, and the P_f reported for a given experimental period was the average of the three individual collections.

Experimental Protocols. Once the IMCD was mounted on concentric pipettes, perfusion was initiated, and the bath temperature was raised to 37°C over a period of 10–15 min. After an equilibration period of 30–35 min, the sampling procedure for the control period began. After three collections were taken in this period, an experimental agent was added to the bath, and the sampling procedure was repeated after 15–20 min of equilibration. Other agents were added in subsequent periods followed by the equilibration time and the sampling procedure.

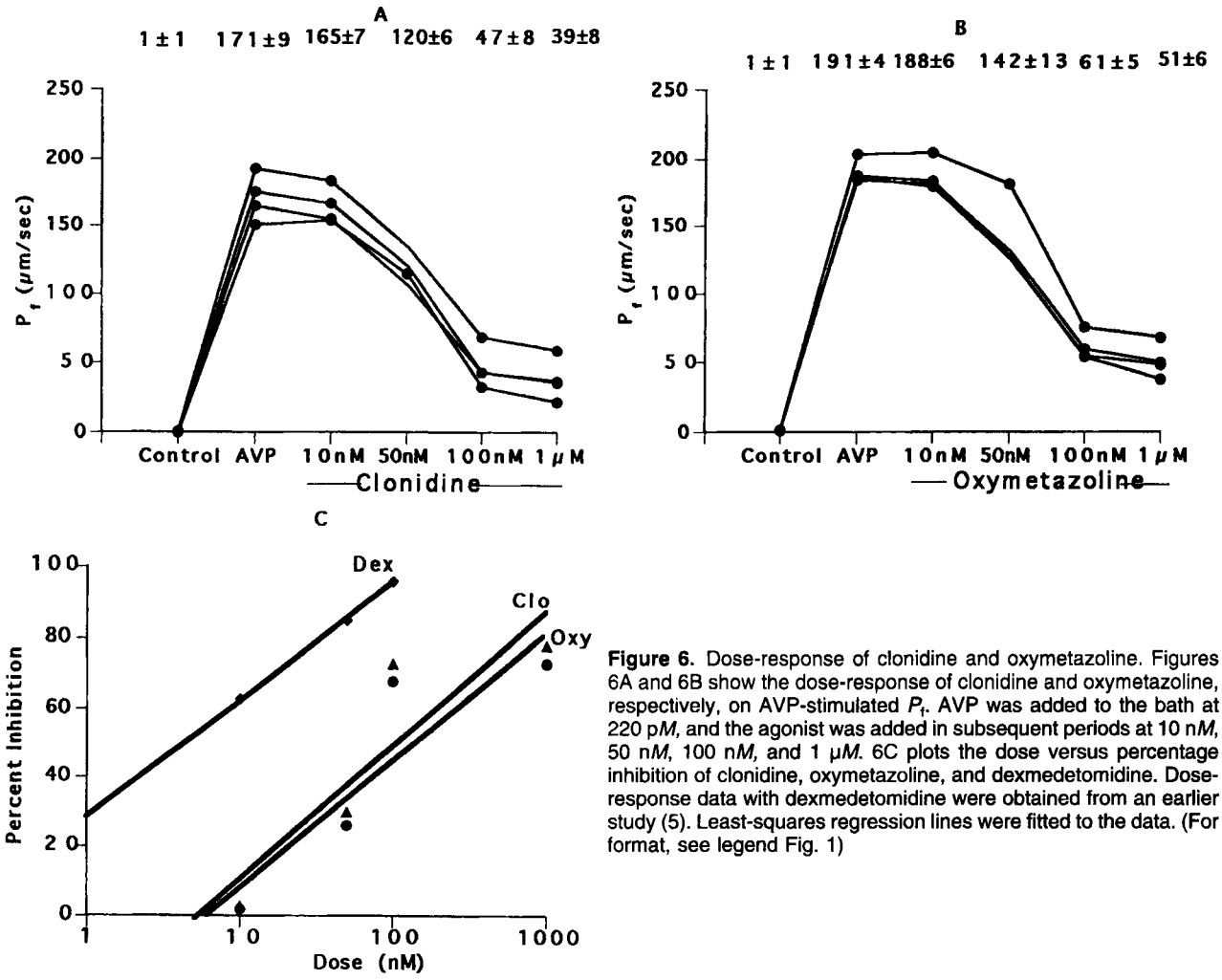

Figure 6. Dose-response of clonidine and oxymetazoline. Figures 6A and 6B show the dose-response of clonidine and oxymetazoline, respectively, on AVP-stimulated P_f. AVP was added to the bath at 220 p*M*, and the agonist was added in subsequent periods at 10 n*M*, 50 n*M*, 100 n*M*, and 1 μ*M*. 6C plots the dose versus percentage inhibition of clonidine, oxymetazoline, and dexmedetomidine. Dose-response data with dexmedetomidine were obtained from an earlier study (5). Least-squares regression lines were fitted to the data. (For format, see legend Fig. 1)

Experiments were designed to determine if either an α_2 or imidazoline agonist would inhibit AVP-stimulated P_f and if an antagonist would reverse the inhibition. The sequence of a given protocol is shown on the abscissa of the graphs in the Results. AVP was used at 220 p*M*, which significantly increased P_f and was consistent with other studies involving the mechanism of water permeability.

In most experiments, agonists and antagonists were used at 100 n*M*. In dose-response protocols of two previous studies, we found that 100 n*M* dexmedetomidine produced maximal or near maximal inhibition of AVP-stimulated P_f in the rat CCD and IMCD (1, 5). In the present study, dose-response protocols were conducted with clonidine and oxymetazoline, and the results were compared with those of dexmedetomidine.

The agonists that inhibited AVP-stimulated water permeability were tested on cAMP-stimulated water permeability. In these studies, we used the nonhydrolyzable analog 8-chlorophenylthio cAMP (CPTcAMP) at 10^{-4} *M* in lieu of AVP. CPTcAMP was an effective analog in these experiments (15).

Source of Biochemicals. AVP, oxymetazoline, WB4101, and CPTcAMP were purchased from Sigma Chemical Co. (St. Louis, MO). RX821002 and idazoxan were purchased from Research Biochemicals International (Natick, MA). Agmatine, rilmenidine, and BU239 were purchased from Torcris Cookson (Ballwin, MO). Clonidine, prazosin, and yohimbine were kindly provided by Boehringer Ingelheim (Ridgefield, CT). Dexmedetomidine and atipamezole were kindly provided by Dr. Riku Aantaa, Chief of Research, Orion-Farmos Pharmaceutical (Turku, Finland). ^{14}C inulin was purchased from New England Nuclear (Boston, MA).

Statistical Analysis. Data were analyzed with a single-factor ANOVA with repeated measures, and *P*-values between treatments were determined using the SuperAnova statistical package.

Results

Figures 1, 2, and 3 show the results of four α_2-antagonists that were effective at reversing α_2-mediated inhibition of AVP-stimulated P_f.

Figure 1 includes three protocols demonstrating the antagonist effect on dexmedetomidine-induced inhibition of AVP-stimulated P_f. Dexmedetomidine inhibited P_f by 90%–95%. The following antagonists reversed inhibition:

1) yohimbine by 46% (Fig. 1A); 2) RX821002 by 29% (Fig. 1B); and 3) idazoxan by 11% (Fig. 1C). The percentage reversal by each antagonist was statistically significant. Results with atipamezole were reported previously (5).

Figure 2 includes four protocols of the antagonist effect on clonidine-induced inhibition of AVP-stimulated P_f. Clonidine inhibited P_f by 75%–80%. The following antagonists reversed inhibition: 1) yohimbine by 33% (Fig. 2A); 2) RX821002 by 39% (Fig. 2B); 3) idazoxan by 33% (Fig. 2C); and 4) atipamezole by 40% (Fig. 2D). The percentage reversal by each antagonist was statistically significant.

Figure 3 includes four protocols demonstrating the antagonist effect on oxymetazoline-induced inhibition of AVP-stimulated P_f. Oxymetazoline inhibited P_f by 65%–75%. The following antagonists reversed inhibition: 1) yohimbine by 58% (Fig. 3A); 2) RX821002 by 80% (Fig. 3B); 3) idazoxan by 47% (Fig. 3C); and 4) atipamezole by 39% (Fig. 3D). The percentage reversal by each antagonist was statistically significant.

Figure 4 shows that prazosin used at 100 n*M* or 1 μ*M* failed to reverse dexmedetomidine-induced inhibition, and Figure 5 shows that WB4101 at 1 μ*M* failed to reverse dexmedetomidine-induced inhibition.

Figure 6 shows the dose-response of clonidine and oxymetazoline, respectively, on AVP-stimulated P_f. After the AVP period, the agonists were added sequentially to the bath at 10 n*M*, 50 n*M*, 100 n*M*, and 1 μ*M*. The dose-response protocol for dexmedetomidine was reported in an earlier paper (5). Figure 6 plots the dose versus percentage inhibition of dexmedetomidine, clonidine and oxymetazoline.

Figures 7, 8, and 9 show the effects of the imidazoline-selective agents. Figure 7 shows that agmatine at 1 μ*M* did not affect AVP-stimulated P_f. Figures 8A and 8B indicate that 1 μ*M* rilmenidine did not affect AVP-stimulated P_f whereas Figures 8C and 8D show that rilmenidine, when added before AVP, significantly reduced P_f. Figure 9 contains three protocols indicating that BU239 at 1 μ*M* reversed α_2-mediated inhibition of AVP-stimulated P_f.

Figure 10 shows that both clonidine and oxymetazoline inhibited cAMP-stimulated P_f. BU239 at 100 n*M* did not reverse the inhibition in either protocol. Figure 11 shows that rilmenidine did not affect cAMP-stimulated P_f in the way it affected AVP-stimulated P_f (Fig. 8D).

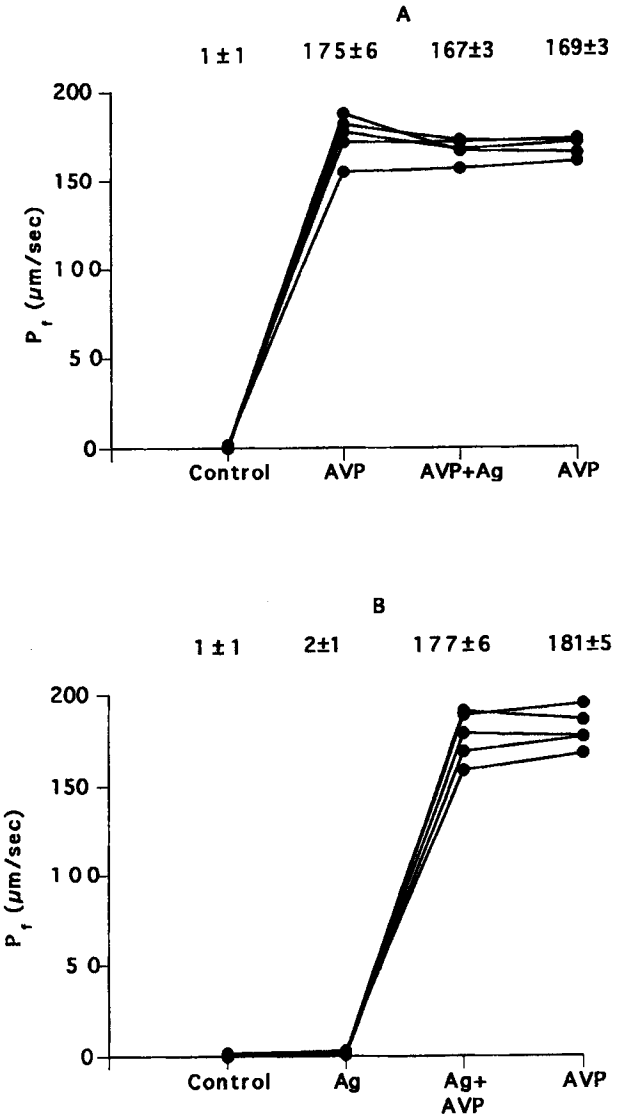

Figure 7. Agmatine did not affect AVP-stimulated P_f. (7A) AVP added to the bath at 220 p*M* increased P_f ($P < 0.001$), and the subsequent addition of agmatine (Ag) at 1 μ*M* did not affect P_f. (7B) Agmatine added after the control period did not affect P_f, and the subsequent addition of AVP increased P_f significantly ($P < 0.001$). (For format, see legend Fig. 1)

Discussion

Elevated plasma AVP levels increase water permeability in the collecting duct thereby increasing water absorption from the kidney. Studies have shown that α_2 agonists inhibit AVP-stimulated P_f in isolated collecting duct nephron segments (1–3, 5, 16). It is well known that AVP increases cellular cAMP levels, and investigators generally agree that α_2 agonists reduce AVP-stimulated transport *via* the inhibition of adenylyl cyclase activity. Chabardés *et al.* (17) and Umemura *et al.* (18) reported that α_2-mediated action *via* clonidine and epinephrine, respectively, inhibited AVP-induced increase in cAMP levels in the rat CCD. Edwards *et al.* (19) and Maeda *et al.* (20) reported the same effect in the rat IMCD.

Interestingly, evidence also indicates that the α_2-mediated inhibition of P_f in the collecting duct involves a cAMP-independent component. Hawk *et al.* (3) reported that in the isolated rat CCD, epinephrine inhibited P_f subsequent to its stimulation by bromoadenosine cAMP, a non-hydrolyzable analog of cAMP. Although this degree of inhibition was small compared to that of epinephrine subsequent to the stimulation of P_f by AVP, it was significant and reversed by yohimbine. Previously, we reported that in the rat IMCD, epinephrine and dexmedetomidine inhibited P_f subsequent to stimulation by CPTcAMP, and atipamezole

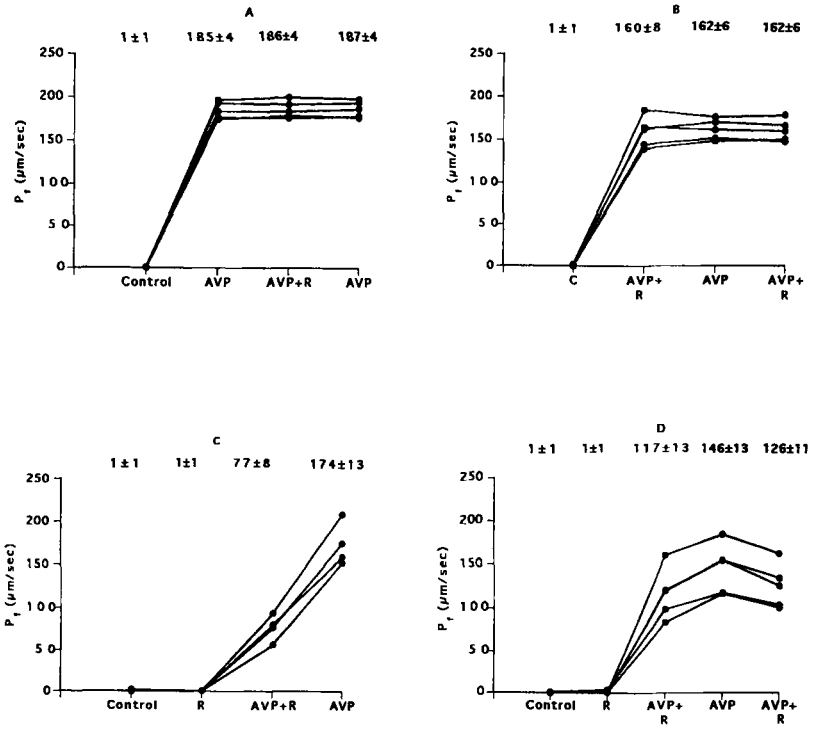

Figure 8. Rilmenidine affected AVP-stimulated P_f when added before AVP. (8 A) AVP at 220 p*M* added to the bath increased P_f ($P < 0.001$), and the subsequent addition of rilmenidine (R) at 1 μ*M* did not affect P_f. (8B) AVP and rilmenidine were added together in the second period where P_f increased significantly from control ($P < 0.001$). The subsequent removal of rilmenidine did not affect P_f. (8C) Rilmenidine was added after the control period without AVP. The subsequent addition of AVP elevated P_f ($P < 0.001$), and the removal of rilmenidine in the fourth period raised P_f further ($P < 0.001$). (8D) the AVP + R period was significantly higher than the previous period ($P < 0.001$). The removal of rilmenidine in the fourth period elevated P_f significantly ($P < 0.01$), and the addition of rilmenidine in the final period decreased P_f significantly ($P < 0.05$). (For format, see legend Fig. 1)

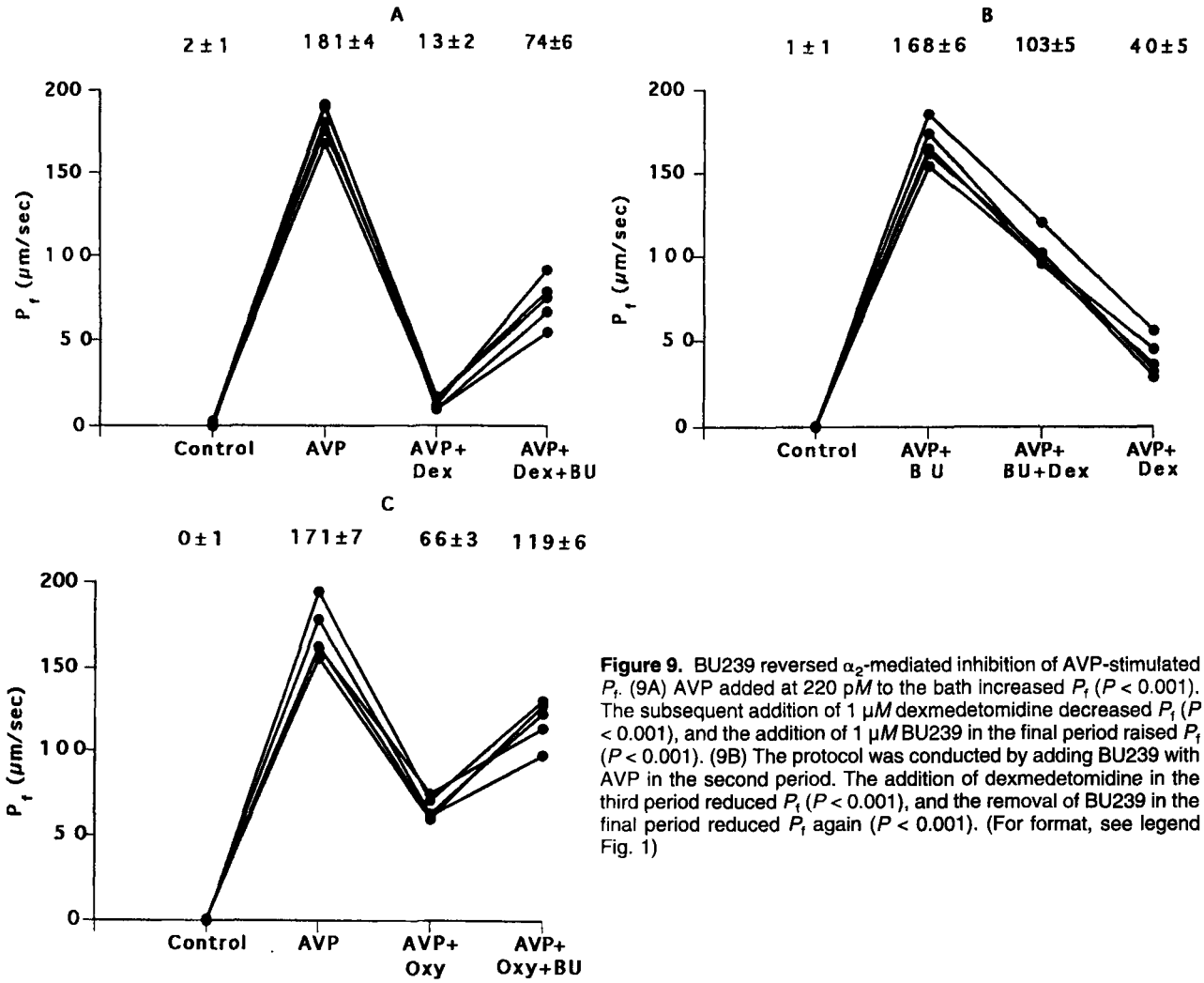

Figure 9. BU239 reversed α_2-mediated inhibition of AVP-stimulated P_f. (9A) AVP added at 220 p*M* to the bath increased P_f ($P < 0.001$). The subsequent addition of 1 μ*M* dexmedetomidine decreased P_f ($P < 0.001$), and the addition of 1 μ*M* BU239 in the final period raised P_f ($P < 0.001$). (9B) The protocol was conducted by adding BU239 with AVP in the second period. The addition of dexmedetomidine in the third period reduced P_f ($P < 0.001$), and the removal of BU239 in the final period reduced P_f again ($P < 0.001$). (For format, see legend Fig. 1)

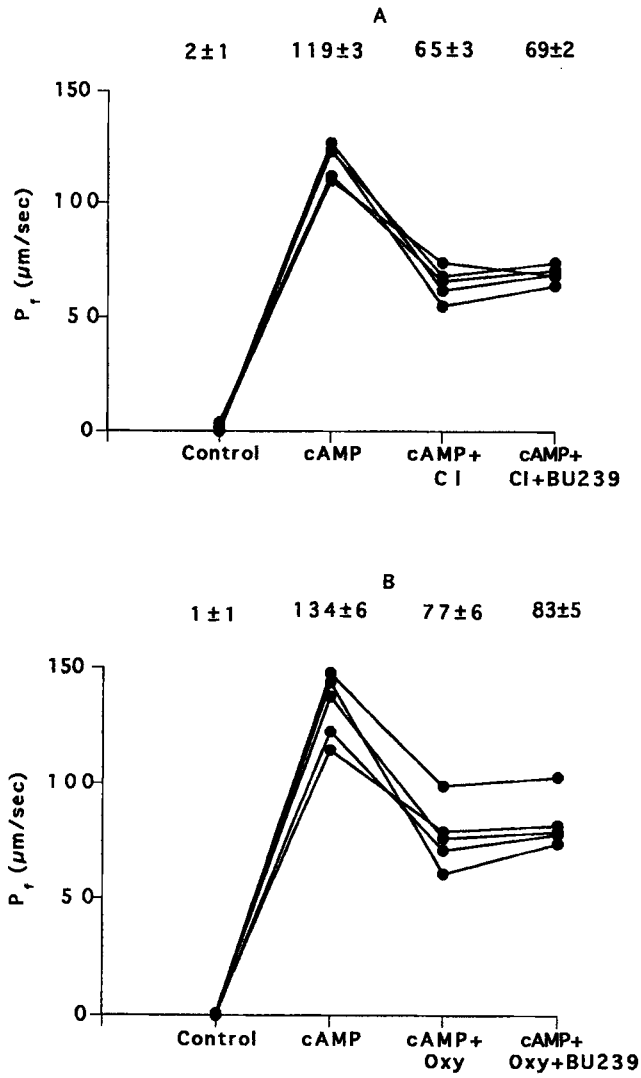

Figure 10. Clonidine and oxymetazoline inhibit cAMP-stimulated P_f. 8-Chlorophenylthio cyclic AMP (cAMP) added to the bath at 10^{-4} *M* increased P_f in both protocols. (10 A) Clonidine or (10B) oxymetazoline added to the bath at 100 n*M* reduced P_f significantly ($P < 0.001$). BU239 at 100 n*M* did not reverse P_f in either protocol. (For format, see legend Fig. 1)

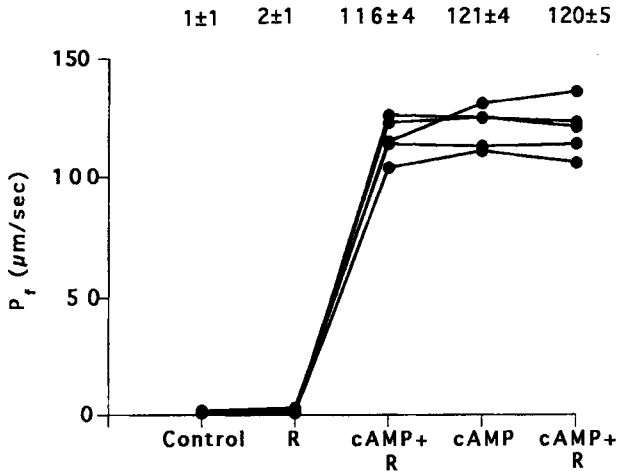

Figure 11. Rilmenidine failed to affect cAMP-stimulated P_f. This is the same protocol as that in Figure 8D except 8-chlorophenylthio cyclic AMP at 10^{-4} *M* was used in lieu of AVP. Rilmenidine at 1 μ*M* did not prevent a maximal stimulation by cAMP as it did with AVP (Fig. 8D). (For format, see legend Fig. 1)

reversed inhibition by both agonists (5). Apparently then, other second messengers and/or other receptors participate in this mechanism.

One well-known effect of α_2 agonists is their ability to reduce blood pressure *via* receptor-mediated action in the central nervous system (21). It is now widely accepted that this hypotensive effect results from α_2 agonists acting on not only α_2 adrenoceptors but also nonadrenergic imidazoline receptors (22). Over the past several years, imidazoline receptors have been studied extensively in numerous tissues, and they have been reported to exist in the rat kidney (23, 24).

Investigators using pharmacological binding techniques have identified agonists and antagonists that distinguish imidazoline receptors from α_2 adrenoceptors and distinguish among the α_2 adrenoceptor subtypes—α_{2A}, α_{2B} and α_{2C}. These subtypes have been identified and cloned (6). The purpose of the present study was to test and compare some of these agents on AVP- and cAMP-stimulated P_f in the rat IMCD. The α_2 agonists used in the study were dexmedetomidine, clonidine, and oxymetazoline. The α_2 antagonists were yohimbine, RX821002 (methoxy-idazoxan), idazoxan, atipamezole, prazosin, and WB4101.

Generally, more antagonists than agonists have demonstrated subtype selectivity. Yohimbine and atipamezole are α_2 antagonists that are nonselective for the α_2 adrenoceptor subtypes (25, 26). RX821002 has been reported to possess selectivity for the α_{2A} subtype (27). Idazoxan is a nonselective α_2 antagonist that also binds to imidazoline receptors and has been used to distinguish between the two (28–30).

We tested these antagonists first with dexmedetomidine, which at 100 n*M* significantly inhibited AVP-stimulated P_f by 85%–95%. Yohimbine, RX821002, and idazoxan significantly reversed the inhibition by 49%, 29%, and 11%, respectively (Figs. 1A, 1B, and 1C). Atipamezole was shown previously to reverse dexmedetomidine-induced inhibition (5). Clonidine inhibited AVP-stimulated P_f by 75%–80%, and the following four antagonists reversed the inhibition: yohimbine produced 33% reversal; RX821002 39%; idazoxan 33%; and atipamezole 40% (Figs. 2A, 2B, 2C, and 2D).

Uhlén and Wikberg (7) reported that oxymetazoline bound with high and low affinity to the α_{2A} and α_{2B} adrenoceptor, respectively, in the rat kidney and that prazosin demonstrated the opposite binding characteristics. WB4101 appears to be selective for the α_{2C} adrenoceptor (25). Our results showed that oxymetazoline significantly inhibited AVP-stimulated P_f by 60%–75%. Yohimbine reversed this inhibition by 58%, RX821002 by 80%, idazoxan by 47%, and atipamezole by 39% (Fig. 3). Interestingly, RX821002 reversed inhibition higher than any agonist-antagonist combination. Figures 4 and 5 show that both prazosin and WB4101 failed to reverse α_2 inhibition of P_f. Thus, based

on the pharmacological properties of these agonists and antagonists, our data suggest that the α_{2A} adrenoceptor plays a role in modulating AVP-stimulated P_f in the rat IMCD.

We conducted dose-response protocols with clonidine and oxymetazoline and compared these results with the dexmedetomidine dose-response protocol conducted in an earlier study (5). The ED_{50} data from Table I indicate that dexmedetomidine is more potent than the other two agonists. No significant differences were obtained from the slope data. This might suggest that all three agonists bind to the same receptor, and dexmedetomidine is more potent due to greater affinity or efficacy, or both. It is also possible that dexmedetomidine binds to more receptors than the other agonists while demonstrating similar dose-response characteristics. Since the data in Figures 4 and 5 show no effect of the α_{2B} antagonist prazosin or the α_{2C} antagonist WB4101, it is reasonable to suspect the involvement of imidazoline receptors.

Figures 7, 8, and 9 show the effects of imidazoline-selective agents on P_f. We began with the putative endogenous imidazoline agonist agmatine, which is a metabolite of arginine decarboxylation (31). Agmatine has been shown to induce diuresis in the rat (31–33). However, our results showed no effect of agmatine on water permeability in the rat IMCD (Fig. 7).

Rilmenidine, another imidazoline agonist, which also affects kidney function in the rat (34, 35), significantly inhibited P_f albeit the inhibition depended on the sequence of experimental periods. Rilmenidine did not affect AVP-stimulated P_f when it was added after AVP (Fig. 8A); however, when added before AVP, rilmenidine prevented a maximal AVP-induced increase in P_f (Fig. 8C). To be certain, we repeated the protocol and observed the same effect, and we also added another period where we again added rilmenidine to the bath with AVP and observed a significant reduction in P_f (Fig. 8D). Rilmenidine produced a different inhibitory profile from that observed with dexmedetomidine, clonidine, or oxymetazoline and thus appeared to inhibit P_f *via* a mechanism separate from the α_2-mediated inhibition of AVP-stimulated P_f. Results on cAMP-stimulation of P_f further demonstrated a different mechanism (see below).

BU239 is a new agent that is highly selective for imidazoline receptors over α_2 adrenoceptors (36, 37). Being uncertain if it acted as an agonist or antagonist, we first tested its potential to inhibit AVP-stimulated P_f and found no effect (results not shown). We then tested the potential antagonist ability of BU239 and found that at 1 μM, it significantly reversed both dexmedetomidine- and oxymetazoline-induced inhibition of AVP-stimulated P_f (Fig. 9).

It was shown earlier that dexmedetomidine inhibited cAMP-stimulated P_f (5). To determine if the clonidine- and oxymetazoline-induced inhibition of AVP-stimulated P_f was dependent on adenylyl cyclase, we tested these agonists on P_f stimulated by the nonhydrolyzable analog 8CPTcAMP in lieu of AVP. Both clonidine and oxymetazoline inhibited cAMP-stimulated P_f (Fig. 10). These findings indicate that inhibition of P_f involves a cellular mechanism more complex than just the inhibition of adenylyl cyclase (i.e., a postcAMP cellular event).

In these cAMP experiments we tested the ability of BU239 in the nanomolar range to reverse inhibition. BU239 at 100 nM failed to reverse either clonidine- or oxymetazoline-induced inhibition. This caused us to question the involvement of imidazoline receptors. Nutt *et al.* (37) reported that BU239 was quite selective for imidazoline receptors in rabbit brain membranes with an inhibitory binding constant (K_i) of about 2 nM whereas K_i for the α_2 adrenoceptors was about 7 μM. It is possible that at higher concentrations BU239 binds to α_2 adrenoceptors, and it is critical to remember that many α_2 and imidazoline compounds demonstrate cross-binding characteristics. Identification of specific receptors requires numerous dose-response studies with highly selective agents.

However, the results noted above with rilmenidine are interesting in that this agonist induced inhibition of AVP-stimulated P_f only when it was applied before AVP. We repeated the protocol with 8CPTcAMP in lieu of AVP. Under these conditions, rilmenidine failed to inhibit P_f (Fig. 11). These results suggested that the rilmenidine-induced effect occurs *via* adenylyl cyclase inhibition (i.e., a precyclic AMP cellular event). Whether rilmenidine acts on imidazoline receptors or *via* another mechanism is not known. Moreover, the cellular mechanism associated with imidazoline receptors is unknown. These results with rilmenidine should lead to further studies to identify the underlying mechanism of this inhibition.

Table I. Dose-Response Data[a]

Agonist	ED_{50} (nM)[b]	95%CI-ED50[c]	Slope[d]	95%CI-Slope[e]
Dexmedetomidine	4.1	1.9–9.0	33.0 ± 5.2	22.3–43.8
Clonidine	105.4	63.5–175.1	38.2 ± 5.4	26.6–49.7
Oxymetazoline	135.5	79.7–230.5	37.0 ± 5.3	25.6–48.4

[a] Data analyzed from dose-response protocols of dexmedetomidine-, clonidine-, and oxymetazoline-induced inhibition of AVP-stimulated P_f (Fig. 6)
[b] ED_{50}: Experimental dose that induced 50% inhibition of AVP-stimulated P_f.
[c] 95% confidence interval of ED_{50} data.
[d] Slope of lines fitted with least squares regression (Fig. 6).
[e] 95% confidence interval of slope data.

In summary, the key new findings obtained from this study are: clonidine and oxymetazoline inhibit AVP- and cAMP-stimulated P_f in the rat IMCD; dexmedetomidine is a more potent inhibitor than either clonidine or oxymetazoline; and the imidazoline-selective agonist rilmenidine inhibits AVP-stimulated P_f *via* a different mechanism than that of the α_2 agonists. In addition, the selective imidazoline agonist agmatine does not affect P_f, and the imidazoline-selective antagonist BU239 at 1 μM but not at 100 nM reverses α_2-inhibition of P_f. Yohimbine, RX821002, idazoxan, and atipamezole reverse α_2-inhibition of AVP-stimulated P_f, whereas prazosin and WB4101 do not. Our data suggest that the α_2- inhibitory mechanism of water permeability in the Wistar rat IMCD is modulated at least in part by α_{2A} adrenoceptors. Although the case for imidazoline receptors is inconclusive, the interesting results with rilmenidine should lead to further mechanistic studies related to that agent.

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1. Rouch AJ, Kudo LH, Hébert C. Dexmedetomidine inhibits osmotic water permeability in the rat cortical collecting duct. J Pharmacol Exp Ther **281**:62–69, 1997.
2. Chen L, Reif MC, Schafer JA. Clonidine and PGE_2 have different effects on Na^+ and water transport in the rat and rabbit CCD. Am J Physiol **261**:F126–F136, 1991.
3. Hawk CT, Kudo LH, Rouch AJ, Schafer JA. Inhibition by epinephrine of AVP- and cAMP-stimulated Na^+ and water transport in Dahl rat CCD. Am J Physiol **265**:F449–F460, 1993.
4. Star RA, Nonoguchi H, Balaban R, Knepper MA. Calcium and cyclic adenosine monophosphate as second messengers for vasopressin in the rat inner medullary collecting duct. J Clin Invest **81**:1879–1888, 1988.
5. Rouch AJ, Kudo LH. α_2-Mediated inhibition of water and urea permeability in the rat inner medullary collecting duct. Am J Physiol **271**:F150–F157, 1996.
6. Bylund DB, Eikenberg DC, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP, Molinoff PB, Ruffolo RR, Jr., Trendelenburg U. International Union of Pharmacology nomenclature of adrenoceptors. Pharmacol Rev **46**:121–136, 1994.
7. Uhlén S, Wikberg JE. Delineation of three pharmacological subtypes of α_2-adrenoceptor in the rat kidney. Br J Pharmacol **104**:657–664, 1991.
8. Bousquet P. Imidazoline receptors: From basic concepts to recent developments. J Cardiovasc Pharmacol **26**(Suppl 2):S1–S6, 1995.
9. Hamilton CA. Imidazoline receptors, subclassification, and drug-induced regulation. Ann N Y Acad Sci **763**:57–65, 1995.
10. Rocha AS, Kokko JP. Permeability of medullary nephron segments to urea and water: Effect of vasopressin. Kidney Int **22**:379–387, 1973.
11. Rocha AS, Kudo LH. Water, urea, sodium, chloride, and potassium transport in the *in vitro* isolated perfused papillary collecting duct. Kidney Int **22**:485–491, 1982.
12. Madsen KM, Tisher CC. Structural-functional relationships along the distal nephron. Am J Physiol **250**:F1–F15, 1986.
13. Sands JM, Nonoguchi H, Knepper MA. Vasopressin effects on urea and H_2O transport in inner medullary collecting subsegments. Am J Physiol **253**:F823–F832, 1987.
14. Al-Zahid G, Schafer JA, Troutman SL, Andreoli TE. Effect of antidiuretic hormone on water and solute permeation, and the activation energies for these processes, in mammalian cortical collecting tubules. J Membr Biol **31**:103–129, 1977.
15. Snyder HM, Noland TD, Breyer MD. cAMP-dependent protein kinase mediates hydrosmotic effect of vasopressin in collecting duct. Am J Physiol **32**:C147–C153, 1992.
16. Krothapalli RK, Suki WN. Functional characterization of the α-adrenergic receptor modulating the hydroosmotic effect of vasopressin on the rabbit cortical collecting tubule. J Clin Invest **73**:740–749, 1984.
17. Chabardés D, Montegut M, Imbert-Teboul M, Morel F. Inhibition of α_2-adrenergic agonists on AVP-induced cAMP accumulation in isolated collecting tubule of the rat kidney. Mol Cell Endocrinol **37**:263–275, 1984.
18. Umemura S, Marver D, Smyth DD, Pettinger WA. α_2-Adrenoceptors and cellular cAMP levels in single nephron segments from the rat. Am J Physiol **249**:F28–F33, 1985.
19. Edwards RM, Gellai M. Inhibition of vasopressin-stimulated cyclic AMP accumulation by α_2-adrenoceptor agonists in isolated papillary collecting ducts. J Pharm Exp Ther **244**:526–530, 1988.
20. Maeda Y, Terada Y, Nonoguchi H, Knepper MA. Hormone and autacoid regulation of cAMP production in rat IMCD subsegments. Am J Physiol **263**:F319–F327, 1992.
21. Evans RG. Current status of putative imidazoline (I_1) receptors and renal mechanisms in relation to their antihypertensive therapeutic potential. Clin Exp Pharmacol Physiol **23**:845–854, 1996.
22. Codd EE, Press JB, Raffa RB. α_2-Adrenoceptors vs. imidazoline receptors: Implications for α_2-mediated analgesia and other noncardiovascular therapeutic uses. Life Sci **56**:63–74, 1995.
23. Ernsberger P, Feinland G, Meeley MP, Reis DJ. Characterization and visualization of clonidine-sensitive imidazole sites in rat kidney which recognize clonidine-displacing substance. Am J Hypertens **3**:90–97, 1990.
24. MacKinnon AC, Stewart M, Olverman HJ, Spedding M, Brown CM. [^{3}H]p-aminoclonidine and [^{3}H]idazoxan label different populations of imidazoline sites on rat kidney. Eur J Pharmacol **232**:79–87, 1993.
25. Blaxall HS, Murphy TJ, Baker JC, Ray C, Bylund DB. Characterization of the α_2C-adrenergic receptor subtype in opossum kidney and in the OK cell line. J Pharmacol Exp Ther **259**:323–329, 1991.
26. Pertovaara A. Antinociception induced by α_2-adrenoceptor agonists, with special emphasis on medetomidine studies. Prog Neurobiol **40**:691–709, 1993.
27. Michel MC, Rump LC. α-Adrenergic regulation of human renal function. Fundam Clin Pharmacol **10**:493–503, 1996.
28. Lauchaud-Pettiti V, Podevin R-A, Chrétien Y, Parini A. Imidazoline-guanidinium and α_2-adrenergic binding sites in basolateral membranes from human kidney. Eur J Pharmacol **206**:23–31, 1991.
29. Michel MC, Brodde O-E, Schnepel B, Behrendt J, Tschada R, Motulsky HJ, Insel PA. [^{3}H] idazoxan and some other α_2-adrenergic drugs also bind with high affinity to a nonadrenergic site. Mol Pharmacol **35**:324–330, 1989.
30. Rangel N, Uhlén S, Wikberg JES. Detomidine binding to guinea pig liver imidazoline receptors (I-receptors) shows marked positive cooperativity. Pharmacol Toxicol **73**:86–90, 1993.
31. Lortie MJ, Novotny WF, Peterson OW, Vallon V, Malvey K, Mendonca M, Satriano J, Insel P, Thomson SC, Blantz RC. Agmatine, a bioactive metabolite of arginine. J Clin Invest **97**:413–420, 1996.
32. Penner SB, Smyth DD. Natriuresis following central and peripheral

administration of agmatine in the rat. Pharmacology **53**:160–169, 1996.
33. Smyth DD, Darkwa FK, Penner SB. Imidazoline receptors and sodium excretion in the rat kidney. Annal N Y Acad Sci **763**:340–352, 1995.
34. Ernsberger PR, Westbrooks KL, Christen MO, Schäfer SG. A second generation of centrally acting antihypertensive agents act on putative I_1-imidazoline receptors. J Cardiovasc Pharmacol **20**(Suppl 4): S1–S10, 1992.
35. Smyth DD, Penner SB. Renal I_1-imidazoline receptor-selective compounds mediate natriuresis in the rat. J Cardiovasc Pharmacol **26**(Suppl 2):S63–S67, 1995.
36. Hudson AL, Chapleo CB, Lewis JW, Husbands S, Grivas K, Mallard NJ, Nutt DJ. Identification of ligands selective for central I_2-imidazoline binding sites. Neurochem Int **30**:47–53, 1997.
37. Nutt DJ, French N, Handley S, Hudson A, Husbands S, Jackson H, Jordan S, Lalies MD, Lewis J, Lione L, Mallard N, Pratt J. Functional studies of specific imidazoline-2 receptor ligands. Ann N Y Acad Sci **763**:125–139, 1995.