Vasopressin Potentiates Mineralocorticoid Selectivity by Stimulating 11β Hydroxysteroid Deshydrogenase in Rat Collecting Duct

Nadia Alfaidy, Marcel Blot-Chabaud, Jean-Pierre Bonvalet, and Nicolette Farman

INSERM U246, Institut Fédératif de Recherches Cellules Épithéliales, Faculté de Médecine X. Bichat, BP 416, 75870 Paris Cedex 18, France

Abstract

Arginine vasopressin (AVP) and corticosteroid hormones are involved in sodium reabsorption regulation in the renal collecting duct. Synergy between AVP and aldosterone has been well documented, although its mechanism remains unclear. Both aldosterone and glucocorticoid hormones bind to the mineralocorticoid receptor (MR), and mineralocorticoid selectivity depends on the MR-protecting enzyme 11β hydroxysteroid deshydrogenase (11-HSD), which metabolizes glucocorticoids into derivatives with low affinity for MR. We have investigated whether the activity of 11-HSD could be influenced by AVP and corticosteroid hormones. This study shows that in isolated rat renal collecting ducts, AVP increases 11-HSD catalytic activity. This effect is maximal at 10^{-8} M AVP (a concentration clearly above the normal physiological range of AVP concentrations) and involves the V2 receptor pathway, while activation of protein kinase C or changes in intracellular calcium are ineffective. The stimulatory effect of AVP on 11-HSD is largely reduced after adrenalectomy, and is selectively restored by infusion of aldosterone, not glucocorticoids. We conclude that this synergy between AVP and aldosterone in controlling the activity of 11-HSD is likely to play a pivotal role in resetting mineralocorticoid selectivity, and hence sodium reabsorption capacities of the renal collecting duct. (*J. Clin. Invest.* 1997. 100:2437–2442.) Key words: sodium reabsorption • mineralocorticoid receptor • aldosterone • kidney • isolated tubules • HPLC

Introduction

In the kidney both arginine-vasopressin (AVP)[1] and corticosteroid hormones contribute to salt and water homeostasis (1). A synergistic action of AVP and aldosterone or deoxycorticosterone acetate to enhance sodium reabsorption has been reported in the collecting duct (1–4). In addition, both glucocorticoid hormones and AVP are required to ensure full efficiency of the urinary concentration–dilution process (5). The mechanism(s) underlying these interactions, however, is(are) largely unknown.

AVP binds to two types of membrane receptors, V1 and V2, which are coupled to distinct second messengers, namely the diacylglycerol-Ca^{2+} and the cAMP-PKA pathways (6), both present in the collecting tubule (CCD). Activation of the V2 pathway results in enhancement of osmotic water permeability and sodium reabsorption. Aldosterone and glucocorticoid hormones bind to intracellular receptors, the mineralocorticoid (MR) and the glucocorticoid (GR) receptors that are coexpressed in the collecting tubule (7), and act as transcription factors to modulate gene expression (8). It has been shown that aldosterone and glucocorticoid hormones display the same affinity for the mineralocorticoid receptor (9). In aldosterone-sensitive cells such as those of the distal parts of the nephron, mineralocorticoid selectivity (9–15) is ensured by the presence of the enzyme 11 β hydroxysteroid dehydrogenase (11-HSD). This enzyme transforms native glucocorticoids, corticosterone, or cortisol (which circulate at plasma concentrations much higher than that of aldosterone) into derivatives (dehydrocorticosterone, cortisone) with very low affinity for the MR, thus protecting MR against illicit occupancy by glucocorticoids.

This transformation maintains the MR free for aldosterone binding and action. On the other hand, since the affinity of dehydrocorticosterone and cortisone for the glucocorticoid receptor is also very low (9), 11-HSD is likely to affect binding and action of glucocorticoids via its own receptor as well.

Two isoforms of 11-HSD have been cloned: 11-HSD1 (16) and 11-HSD2 (17). 11-HSD2 appears as the actual MR-protecting enzyme, with high affinity for glucocorticoids (K_d = 10^{-9} M), an exclusive dehydrogenase activity, and selective localization in aldosterone-sensitive cells (14, 15, 17, 18). In contrast, the ubiquitous 11-HSD1 has low affinity for glucocorticoids and bidirectional (dehydrogenase and reductase) activity (15, 16). 11-HSD1 depends on the cofactor NADP, while 11-HSD2 depends on NAD. Both isoforms are expressed in the kidney. It has been shown that the collecting duct expresses an enzyme form that has all the properties of 11-HSD2 (14, 15, 18), while the properties of the proximal tubule enzyme are similar to those of 11-HSD1 (14, 15, 18).

In view of the interactions of AVP with both aldosterone and glucocorticoids in the distal part of the nephron (1, 5, 19, 20, 21), one can wonder whether AVP could modulate 11-HSD activity. To address this question, we have evaluated the in vitro effect of AVP on 11-HSD in the rat CCD. Few studies have been devoted to investigating 11-HSD activity regulation. No significant effect of aldosterone and corticosterone on 11-HSD activity was detectable in the rat whole kidney (22) or cortical collecting tubule (18), while corticosterone induced 11-HSD activity in toad bladder (23).

Our results show that in vitro exposure to AVP increases 11-HSD activity in rat CCD. This effect is rapid, dose-dependent,

Address correspondence to N. Farman, Inserm U246, Faculté de Médecine X. Bichat, BP 416, 75870 Paris Cedex 18-France. Phone: 33-1-4485-6323; FAX: 33-1-4229-1644; E-mail: farman@bichat.inserm.fr

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1. *Abbreviations used in this paper:* 11-DHC, 11-dehydrocorticosterone; 11-HSD, 11β hydroxysteroid dehydrogenase; ADX, adrenalectomy; AVP, arginine vasopressin; CCD, cortical collecting duct; GR, glucocorticoid receptor; MR, mineralocorticoid receptor.

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and is mediated through the V2 receptor and adenylate cyclase pathway. It is potentiated by aldosterone, while glucocorticoid hormones are ineffective. This finding requires integration in the cascade of events that contributes to ensure mineralocorticoid selectivity in aldosterone-sensitive cells. AVP-related enhancement of mineralocorticoid selectivity could significantly contribute to optimize sodium reabsorption.

Methods

Animals. Experiments were performed in control or adrenalectomized male Sprague Dawley rats (180–200 g body wt). Animals were fed a standard diet and had free access to tap water. Adrenalectomy (ADX) was performed after ether anesthesia. ADX rats had access to 0.9% NaCl for drinking. 5 d after adrenalectomy, some animals received steroid hormones via osmotic minipumps (Alzet 2001; Alza Corp, Palo Alto, CA), delivering 10 μg/100g body wt/d aldosterone, dexamethasone, or corticosterone for 3.5 d.

Microdissection of CCD. Rats were anesthestized with pentobarbital sodium (5 mg/ 100 g body wt), and 1 ml blood was collected from the vena cava (to determine plasma aldosterone concentration). Kidneys were perfused via the aorta with an ice-cold rinsing solution containing (in mM) 137 NaCl, 5 KCl, 0.8 $MgSO_4$, 0.33 Na_2HPO_4, 0.44 KH_2PO_4, 1 $MgCl_2$, 1 $CaCl_2$, 5 D-glucose, and 10 Tris (hydroxymethyl aminomethane) HCl, pH 7.4, followed by perfusion of collagenase solution (the same solution to which 0.1% collagenase [Serva, Heidelberg, Germany] 0.7 U/mg, was added). At the end of the perfusion, kidneys were removed and thin pyramid pieces were incubated at 30°C for 1 h in the same collagenase solution.

Dissection of CCD was then performed at 4°C under a stereomicroscope in the dissection solution (similar to the perfusion solution except for the absence of collagenase and addition of 0.1% BSA; Sigma Chemical Co., St. Louis, MO). CCDs were isolated as previously described (18), and tubular length was measured using a millimeter scale placed under the microdissection dish.

Determination of 11-HSD activity. Intact tubular segments of CCD (3 mm per assay) were transferred in a minimal volume (1 μl) of microdissection solution, preincubated for 15 min in the presence of 1 mM 3-isobutyl-1-methyl-xanthine (IBMX), an inhibitor of phosphodiesterase, to prevent degradation of endogenous cyclic AMP, and then incubated (10 μm final volume) for 10 min (i.e., in conditions of initial velocity of enzymatic activity [18]) with [^{3}H]corticosterone (1, 2, 6, 7, [^{3}H]corticosterone, sp act: 2.70 TBq/mmol; Amersham Corp., Arlington Heights, IL) in the absence or presence of 10^{-8} M AVP. Experiments have been performed at saturation of enzymatic activity, i.e., at 10^{-6} M [^{3}H]corticosterone (18), by diluting 100-fold the stock [^{3}H]corticosterone with unlabeled corticosterone. Preliminary experiments showed that an effect of AVP could be detected only in intact tubules (the effect was absent in permeabilized tubules, probably because of disruption of receptor-G protein interactions). The effect of several agents was tested: PMA (10^{-6} M), an activator of PKC; A23187 (a calcium ionophore, 10^{-6} M) incubated in the presence of a low (10^{-9} M) or high (10^{-3} M) concentration of Ca^{2+} ; the V2 agonist dDAVP (1-desamino-8-D-arginine vasopressin, 10^{-8} M); and 8-Br cAMP (2.1^{-4} M), a permeant analog of cAMP. All incubations were performed at 37°C for 10 min, except in the experiments designed to examine the time course of the effect of AVP on 11-HSD activity. For all incubations, 1 mM of the cofactor NAD was used, although it should not be involved in the reaction since tubules have not been permeabilized. The reaction was stopped by transferring the samples at 4°C and adding 90 μl mobile-phase HPLC (methanol/H_2O:1/1) containing 10^{-4} M unlabeled corticosterone and 11-dehydrocorticosterone (11-DHC) (Sigma Chemical Co.) as internal standards. Samples were stored at −20°C up to HPLC analysis.

HPLC analysis. A reversed-phase column Novapak C18 (4 μm; Waters Associates, Milford, MA) with precolumn (C18, 5 μm; Waters Associates) was used. Samples were injected onto the column and eluted isocratically with the mobile phase (methanol/H_2O:1/1), 1 ml/min, with the elution profile of unlabeled standards monitored by absorbance at 240 nm (Beckman Gold HPLC System; Beckman Instruments, Gagny, France). Fractions of 0.5 ml were collected every 30 s (Retriever III; Isco Inc., Lincoln, NE) in counting vials containing 3 ml scintillation fluid (Optiphase; Packard Instruments, Inc., Meriden, CT) for 10 min. The radioactivity was counted in a liquid scintillation counter (Rackbeta; LKB Instruments Inc., Gaithersburg, MD). For each sample, the profile of eluted radioactivity was superimposed to the elution profile of unlabeled corticosterone and 11-DHC. Results are expressed as femtomoles of the metabolite produced per 3 mm and per 10 min (fmol/3 mm/10 min).

Statistical analysis. Data are given as mean±SEM (*n*, number of samples). Statistical analysis was performed using a two-tailed Student's *t* test for independent means, or a *t* test for paired values.

Results

Effect of AVP on 11-HSD activity. CCD from normal rats was preincubated or not with IBMX for 15 min, and then incubated for 10 min in the absence or presence of 10^{-8} M AVP. As shown in Fig. 1, AVP significantly increased 11-HSD activity by ∼ 85%, only when tubules were preincubated with IBMX. IBMX alone had no effect on 11-HSD activity. In further experiments, CCDs were systematically preincubated with IBMX.

Characteristics of AVP-induced increase in 11-HSD activity in CCD from normal rats. To examine the time course of AVP action, 11-HSD activity of CCD was measured after different incubation times (0–10 min) in the absence or presence of 10^{-8} M AVP (after preincubation with IBMX). Results are given in Fig. 2 *A*. Values obtained in the presence of AVP were higher than those in the absence of AVP at any time, and the difference became significant at 4 min. Thus, the effect of AVP appears to be very fast, intervening in < 4 min.

The dose-dependency of AVP effect was measured after a 10-min incubation in the presence of 10^{-10}–10^{-6} M AVP (after preincubation with IBMX). Results are illustrated in Fig. 2 *B*. When compared with the control condition (without AVP), an increase in 11-HSD activity was observed from 10^{-10} M that was maximal at 10^{-8} M.

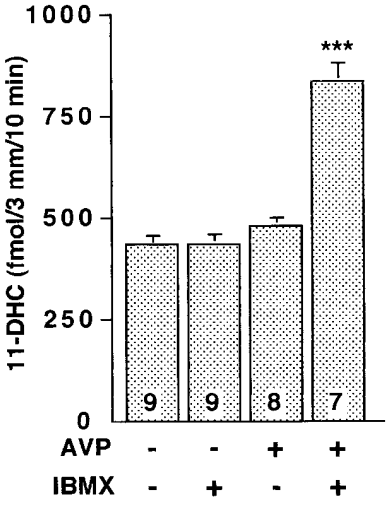

Figure 1. Effect of AVP on 11-HSD catalytic activity. 11-HSD activity is expressed as femtomoles of 11-dehydrocorticosterone (DHC) produced/3 mm tubule/10 min incubation time. CCDs from control rats were incubated in the presence of [^{3}H]corticosterone and 10^{-8} M AVP, preceeded or not by 15 min preincubation in the presence of 1 mM IBMX. Bars are means±SE. Numbers in bars are number of determinations. ***$P < 0.001$ (unpaired *t* test).

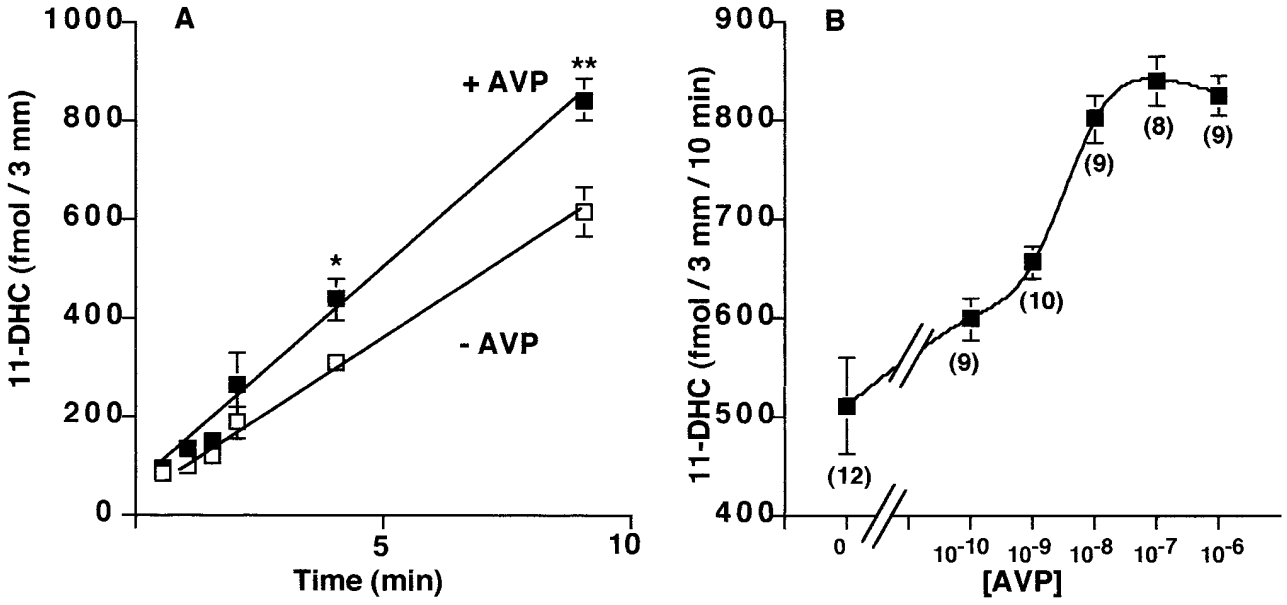

Figure 2. Time course and dose dependency of AVP effect on 11-HSD activity in control rats. (*A*) CCDs were incubated 0.5–10 min with [^{3}H]corticosterone, in the presence or absence of AVP (10^{-8} M) and IBMX. Data are means±SE (each point is the mean of three to four independent determinations). $*P < 0.05$; $**P < 0.02$ (unpaired *t* test). (*B*) CCDs were incubated for 10 min with [^{3}H]corticosterone in the presence of AVP (10^{-10}–10^{-6} M). Data are means±SE of 9–12 determinations.

It has been well documented (6) that AVP acts via two different receptors (V1 and V2) that control the phospholipase C and the adenylate cyclase pathways, respectively. These two receptors are present in the CCD. To determine the type of receptor implied in 11-HSD activity stimulation, we examined the effect of the specific V2 receptor agonist dDAVP. Activity of 11-HSD was mesured in the absence and in the presence of 10^{-8} M AVP or dDAVP (after preincubation with IBMX) (Fig. 3 *A*); dDAVP reproduces the effect of AVP, i.e., stimulation of 11-HSD activity. Thus, it is likely that the effect of AVP on 11-HSD activity depends on interaction of the hormone with V2 receptors.

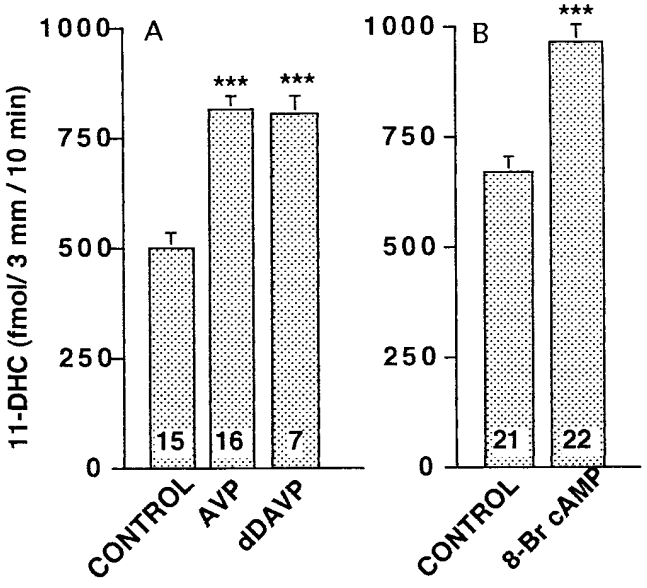

Figure 3. Effects of dDAVP and 8-bromo cyclic AMP on II-HSD activity. Both dDAVP (10^{-8} M), a V2 agonist, and 8-bromo cyclic AMP (2×10^{-4} M) reproduced the effect of AVP on 11-HSD activity in CCD of control rats. Bars are means±SE. Numbers in bars are number of determinations $***P < 0.001$ (unpaired *t* test).

Since the effect of AVP via V2 receptors is mediated by an increase in intracellular cAMP, we have examined whether 8-bromo cyclic AMP (a permeant analog of cAMP) was able to reproduce the effect of AVP. Fig. 3 *B* shows that 10-min incubation of CCD in the presence of 2.10^{-4} M 8-Br cAMP (after preincubation with IBMX) induces a significant increase (44%) of 11-HSD activity.

Activation of the V1 pathway is followed by an increase in intracellular concentration of Ca^{2+} and stimulation of PKC (6). To determine whether interaction of AVP with V1 receptor influences 11-HSD activity, we examined the effect of PMA, a PKC activator, and the influence of the calcium ionophore A23187 in the presence of high (10^{-3} M) or low (10^{-9} M) concentrations of Ca^{2+}. Results are given in Fig. 4. As compared with the control condition, neither activation of PKC nor the imposed variations of intracellular Ca^{2+} concentrations modified 11-HSD activity. This result strongly suggests that the V1 pathway is not involved in the effect of AVP on 11-HSD activity.

Interaction between AVP and corticosteroid hormones on 11-HSD activity. A putative synergistic action between AVP and aldosterone has been investigated. As shown in Fig. 5, AVP (after preincubation with IBMX) significantly increased 11-HSD in CCDs of both ADX and control (normal) rats. The effect, however, was of reduced magnitude in ADX rats (+16%), whereas an increase of ~ 43% was observed in control rats.

Since ADX results in depletion in both gluco- and mineralocorticoid hormones, an experimental series was performed to determine which class of steroid hormones was implicated in

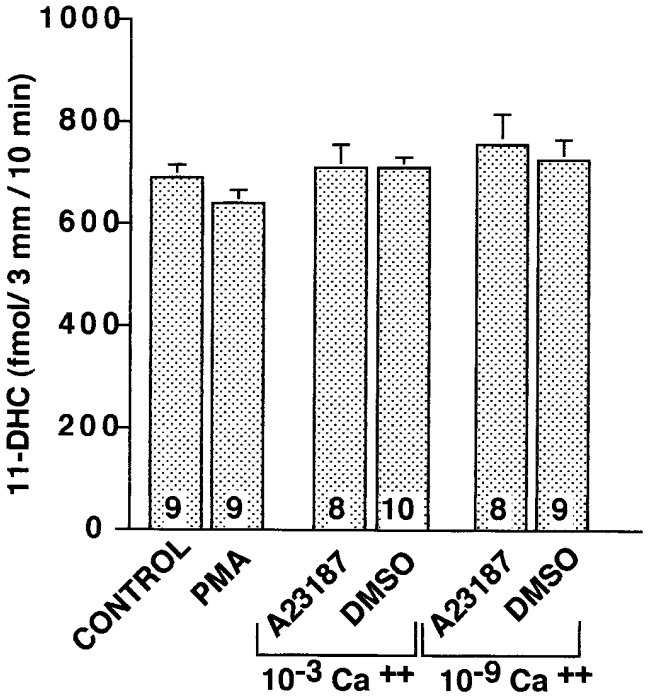

Figure 4. Lack of effects of PMA or changes in intracellular calcium (Cai) on 11-HSD activity. PMA, a stimulator of PKC, and high or low levels of Cai (tubules incubated in the presence of the calcium ionophore A 23183) failed to influence 11-HSD activity in CCD of control rats. Bars are means±SE; numbers in bars are number of determinations.

the potentiation of AVP effect (after preincubation with IBMX). To this purpose, 11-HSD activity was measured in CCD of ADX rats and of ADX rats receiving aldosterone, dexamethasone, a synthetic glucocorticoid, or corticosterone, the natural glucocorticoid in the rat. Fig. 6 shows that glucocorticoid hormones are ineffective in modifying 11-HSD activity in th absence as well as in the presence of AVP. By contrast, aldosterone induced a clear and significant increase in the AVP effect. Aldosterone alone also clearly increased 11-HSD activity in CCD, even in the absence of AVP.

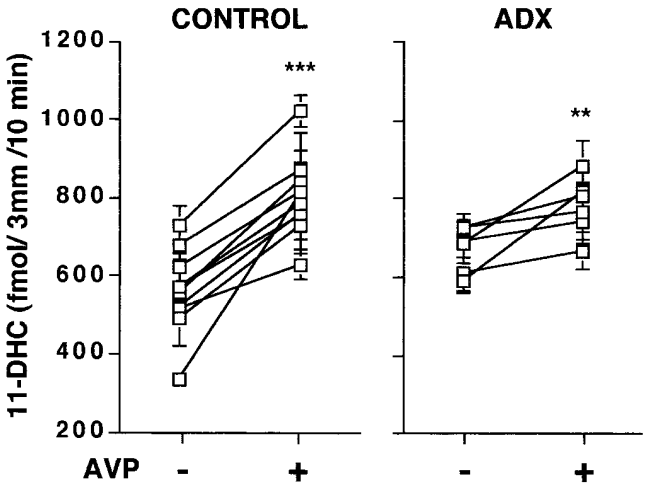

Figure 5. Influence of steroid status on the effect of AVP on 11-HSD activity. The effect of 10^{-8} M AVP was evaluated in CCD originating from ten normal (control) and six ADX rats. Each point is the mean value of 4 to 13 determinations in the same animal. *Solid lines* join mean values of data obtained (±AVP) in the CCDs from the same animal. $^{**}P < 0.01$, $^{***}P < 0.001$, paired *t* test.

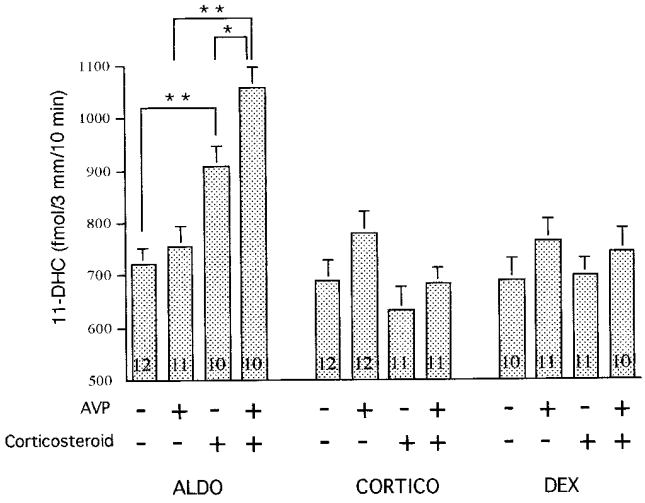

Figure 6. Steroid specificity of the potentiation of AVP on 11-HSD activity. ADX rats were infused with aldosterone (*ALDO*), the natural glucocorticoid corticosterone (*CORTICO*), or the synthetic glucocorticoid dexamethasone (*DEX*); isolated CCDs were treated or not with AVP. Bars are means±SE, numbers in bars are number of determinations. Only aldosterone was effective to potentiate AVP action. $^{*}P < 0.05$; $^{**}P < 0.01$ (unpaired *t* test).

Discussion

These results demonstrate that AVP increases 11 HSD catalytic activity in the rat collecting duct. This increase (~ 50%) occurs within a few minutes. Such a short delay has been consistently reported for other effects of the hormone in the kidney, such as cAMP production (24), modulation of transepithelial ion or water transport (1, 3, 25), or induction of prostaglandin synthesis (26). The dose dependence of the AVP-induced increase in 11-HSD activity (10^{-10}–10^{-8} M) is in partial accordance with previous reports on the in vitro effects of the hormone on cAMP, which saturates at 1 nM AVP (27, 28, 29) on protein kinase activity (30), or on prostaglandin production (26) in the collecting tubule, all of which are maximal at 10^{-8} M. It should be noted that an AVP concentration of 1 nM or higher plus IBMX was required to produce a significant elevation in 11-HSD activity. This high concentration stands in marked contrast to the normal physiological range of AVP concentration (1–100 pM) that produces a maximal physiological effect both in vivo and in isolated CCD segments perfused in vitro. The reason for the difference in sensitivity between the in vitro preparations of isolated tubules (including collagenase incubation) and the isolated perfused CCD or the in vivo tissue remains to be determined, but it might relate to the effects of the preparative procedures on tissue sensitivity to AVP.

Several convergent arguments suggest that the V2 receptor mediates the observed AVP effect. dDAVP, a V2 exclusive agonist, reproduces the AVP-induced increase in 11-HSD ac-

tivity, cAMP, the effector of the V2 pathway, mimics AVP action, and changes in the intracellular Ca^{2+} concentration as well as stimulation of the PKC (both effectors of the V1 pathway) did not affect 11-HSD activity.

The increase in 11-HSD observed in our study is likely to correspond to that of the NAD-dependent 11-HSD2, the MR-protecting enzyme (3, 14, 17, 18), since we (18) and others (14) have previously shown that 11-HSD activity in cells of collecting tubule is an exclusively NAD-dependent dehydrogenase, while cells of the proximal tubule express a bidirectional NADP-dependent enzyme, presumably 11-HSD1.

The AVP-dependent stimulation of 11-HSD activity was lower in ADX rats than in adrenal-intact animals; indeed the observed increase was only 16% in ADX as compared with 43% in intact rats ($P < 0.005$, t paired test). This result clearly indicates that corticosteroid hormones potentiate AVP action. Experiments in ADX animals compensated with corticosteroid hormones demonstrate that the potentiating effect depends on aldosterone, not glucocorticoids. Noticeably, aldosterone alone significantly increased 11-HSD activity independently of AVP.

Synergism between AVP and mineralocorticoid hormones on sodium reabsorption in the renal collecting duct has been well documented, particularly by the group of Schafer (1, 3, 19). A synergistic action of physiological concentrations of AVP and aldosterone, or AVP and DOCA on sodium transport, has been demonstrated by electrophysiological and isotopic methods on isolated rat CCD (19). Similar results were obtained with vasotocin and aldosterone in A6 cells derived from the *Xenopus laevis* kidney (31, 32). Vasopressin-dependent stimulation of adenylate cyclase has been shown to depend on the mineralocorticoid status in rat CCD (20). In mouse CCD, it has been demonstrated that the existence of an AVP-dependent activation of Na,K-ATPase reserve pool requires the presence of aldosterone (21). Full efficiency of the urinary concentration dilution process, which is under the control of AVP, requires the concomitant action of glucocorticoids: hydroosmotic permeability of the rabbit CCD has been shown to be synergistically increased by AVP and aldosterone (5). In addition, it has been recently demonstrated that both aldosterone and glucocorticoids are effective in controlling sodium reabsorption in the collecting duct via the MR and also the GR (33, 34, 35).

These multiple interactions between (at least) three hormonal systems, all of them involved in sodium and/or water reabsorption, point out on the potential interest of a unique enzymatic system modulating them in a coordinated manner.

The crucial role of 11-HSD in mineralocorticoid selectivity must be integrated into a complex multifactorial chain of events that all contribute to this process. More than 90% of glucocorticoids in extracellular fluids are bound to corticosteroid binding globulin (CBG); variations of CBG levels and of glucocorticoid binding to this protein could thus directly influence the availability of free glucocorticoids for binding to the MR, modifying the relative proportion of aldosterone and glucocorticoids available for binding to this receptor (36). The enzyme 11-HSD plays a major role in reducing the concentration of free glucocorticoid hormones in aldosterone target cells. It is likely, however, that a small proportion of glucocorticoids escapes from metabolism. Interactions with MR, then, will be important.

Despite the equivalent affinity of MR for aldosterone and glucocorticoid hormones (37), the nature of the ligand that occupies MR modifies its properties. Importantly, it has now been demonstrated that glucocorticoids and aldosterone exert differential effects on the MR. The velocity and efficiency of nuclear translocation of the ligand–MR complexes are lower with glucocorticoids than with aldosterone as ligand (38). Interaction of MR with aldosterone is much more stable than with glucocorticoids (37). Importantly, the transactivation efficiency of MR is also ligand-dependent; aldosterone is fully efficient as a ligand at concentrations 100-fold lower than those required for glucocorticoids (37, 39). Any change in 11-HSD activity will modify the amount of free glucocorticoids in the cell, influencing the relative proportion of MR-aldosterone and MR-glucocorticoid complexes, thus displacing the equilibrium of subsequent steps towards a new functional status. Even a relatively small enhancement in 11-HSD activity such as that elicited by AVP may lead to a major shift in receptor transactivation efficiency and potentiation of aldosterone action. Concomitantly, this shift will influence the interactions of MR with other partners. Corticosteroids receptors may bind to DNA either as homo or heterodimers with distinct transactivation properties (40). Futhermore, differential interactions of MR with associated nuclear proteins (transcription factors such as c-Jun and c-Fos) may also intervene (41). In this context, the relative proportion of MR- and GR-liganded complexes, which depends at least partially on 11-HSD, is likely to influence the relative proportion of homo and heterodimers available for DNA binding. This, in turn, could modulate the intensity (and perhaps the nature) of the hormonal response.

Since 11 dehydro-derivatives of natural glucocorticoids have negligible affinity for the GR (9), the consequence is that in addition to an enhancement of aldosterone binding to MR, 11-HSD activity is expected to dampen or even supress glucocorticoid effects via the GR in cells expressing both MR and GR, (such as those of the renal collecting duct) (7). Thus, any increase in 11-HSD activity due to AVP is likely to reduce, and any decrease enhance, glucocorticoid action in the cell. Consequently, the amounts of occupied GR will vary, modifying the MR-GR ratio in the nucleus and the subsequent level or nature of the hormonal response.

On the whole, considering (*a*) the renal interactions among AVP, aldosterone, and glucocorticoids; (*b*) the key role of 11-HSD in the control of both mineralo- and glucocorticoid action; and (*c*) the stimulating effect of AVP on 11-HSD2 activity, 11-HSD appears to be a pivotal factor responsible for integrated cellular regulation by these three hormonal systems.

In conclusion, we have demonstrated that AVP stimulates 11-HSD activity in the renal collecting tubule of the rat via the V2 receptor pathway. It is suggested that this mechanism contributes significantly to the integrated cellular response to AVP and corticosteroid hormones.

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