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# COMMENTARY

# ANOMALOUS ANTIDIURETIC ACTIVITY OF ANTIDIURETIC HORMONE ANTAGONISTS

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## *A brief overview of ADH and ADH antagonists*

Vasopressin (antidiuretic hormone, ADH§) plays a primary role in the regulation of renal water excretion and a secondary role in the regulation of cardiovascular function in mammals. Vasopressin exerts its actions through two general classes of vasopressin receptors: $V_2$ receptors which are linked to adenylate cyclase metabolism and regulate the water permeability of terminal renal epithelia; and $V_1$ receptors which are linked to phosphatidylinositol metabolism and mediate contraction of smooth muscle in peripheral vasculature and glucose turnover in hepatocytes [1]. Representative $V_2$ and $V_1$ receptors have been cloned and sequenced [2–4]. Both receptor subtypes include seven membrane-spanning domains, but are otherwise not closely related in structure. $V_2$ and $V_1$ receptors are located on the surface membranes of their respective target cells; both receptors bind vasopressin and are subsequently internalized as vasopressin/receptor complexes through a process of receptor-mediated endocytosis [5]. The significance of internalization of vasopressin/receptor complexes is unknown, but is thought to play a role in the regulation of the density of $V_2$ and $V_1$ receptors and, hence, to contribute to the regulation of the sensitivity of the target cell receptor/effector pathways [5, 6]. The separate antidiuretic ($V_2$) and pressor ($V_1$) actions of vasopressin have long been surmised to interact physiologically to regulate body fluid homeostasis. Recently, however, $V_1$-like receptors have been reported to co-exist with $V_2$ receptors in renal collecting tubules and ducts [7, 8], and $V_2$-like receptors have been reported in vascular/endothelial tissues [9, 10], raising the possibility of direct molecular interactions between the two receptor pathways.

In the last 25 years there has been an explosion of vasopressin analogs, including the discovery of relatively specific agonists and antagonists of $V_2$ and $V_1$ receptors [11, 12]. Arginine vasopressin (AVP) has approximately equivalent antidiuretic and pressor agonist activities, 1-desamino-8-D-arginine vasopressin (dDAVP) is a highly specific antidiuretic agonist, and 8-ornithine vasopressin is a relatively selective pressor agonist [1]. Most of the vasopressin antagonists studied to date were discovered by or derived from the pioneering work of Drs. M. Manning and W. H. Sawyer who reported pressor antagonists in 1978 [13], followed by ADH antagonists in 1981 [14]. With the development and application of receptor binding, adenylate cyclase and inositol phosphate, and epithelial (water flux) and smooth muscle (pressor) assays, the $V_2$ and $V_1$ receptor affinities, activities, and potencies of many of these antagonists have been characterized [11, 15].

The first unambiguously diuretic ADH antagonists were derived from pressor antagonist structures and added $V_2$ antagonist activity to $V_1$ antagonist activity in one molecule; hence, these compounds were dual $V_1/V_2$ receptor antagonists ($V_2$:$V_1$ potency = 0.3:1) [16]. Subsequently, relatively selective ADH antagonists were found ($V_2$:$V_1$ potency = 39:1) [16]. Results of *in vitro* studies with these compounds in amphibian, rodent, canine, primate and human tissues were entirely consistent with the results of *in vivo* studies in rats and a new class of selective water-diuretic (aquaretic) agents based upon antagonism of renal $V_2$ receptors was proposed [17–19]. However, in 1983 *in vivo* inter-species response differences were reported which could not be explained on the basis of *in vitro* findings [20]. In subsequent studies conducted over the remainder of the decade it emerged that: (1) of all the ADH antagonists shown to be aquaretic in rats, only a small subset of the most potent of those compounds (including SK&F 101926 and SK&F 105494) were aquaretic in dogs [21, 22]; (2) of those compounds active in both rats and dogs an even smaller subset (including SK&F 105494) were aquaretic in non-human primates [23, 24]; and (3) neither SK&F 101926 nor SK&F 105494 was aquaretic in humans [25]. Of the compounds that failed to demonstrate aquaresis in non-rodent species, all consistently demonstrated potent and effective $V_2$ receptor antagonist activity and lack of intrinsic agonist activity in all *in vitro* test systems; however, all produced antidiuresis in one or more non-rodent species *in vivo*. Much has been written about (and little resolved) concerning the paradoxical antidiuretic effects of ADH

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§ Abbreviations: ADH, antidiuretic hormone; $V_2$ and $V_1$ receptors, adenylate cyclase- and phosphatidylinositol-coupled vasopressin receptors, respectively; AVP, arginine-8-vasopressin; dDAVP, 1-desamino-8-D-arginine vasopressin; and DI, diabetes insipidus.

antagonists [26]. This commentary reviews one aspect of the antidiuretic effects of ADH antagonists—the antidiuretic effect associated with multi-day infusion or repeat-daily injection of ADH antagonists in rats—and suggests that the results of these studies provide insight into the paradox.

*Antidiuretic effects of ADH antagonists in rats*

Several groups have infused rats, using miniature osmotic infusion pumps (Alzet, Alza Corp., Palo Alto, CA), with ADH antagonists [11, 27–29]. In our laboratory, we were attempting to demonstrate "chemical diabetes insipidus" by infusing the ADH antagonist $d(CH_2)_5Tyr(Et)^2Val^4AVP$ subcutaneously into vasopressin-replete (Sprague–Dawley) rats. The animals were housed in glass metabolism cages and time-lapse cinematography was used to record urine flow rate. After an initial diuretic response, urine flow rate progressively abated until after a few days no diuresis was evident. Hypothesizing that reflex release of endogenous vasopressin had occurred, we repeated the experiment taking advantage of a strain of rats unable to elaborate competent vasopressin (the homozygous Brattleboro strain diabetes insipidus rat, DI rat [11, 28]). We prepared DI rats with minipumps containing vasopressin and, having established a stable antidiuresis in these rats, gave the rats a second minipump containing the ADH antagonist. Again, after an initial diuresis, urine flow progressively abated. We continued the experiment until the vasopressin-containing minipumps were theoretically exhausted, expecting to observe a rapid restoration of the basal DI condition. However, no such restoration occurred and the DI rats remained antidiuretic. Thoroughly confused, we retreated and began a systematic study of the effects of subcutaneous infusions of ADH agonists and antagonists in vasopressin-replete (Sprague–Dawley and Long Evans) and DI rats and obtained the now familiar results illustrated in Figs. 1 and 2. We and others have infused the dual $V_1/V_2$ antagonists $d(CH_2)_5D\text{-}Tyr(Et)^2Val^4AVP$ [30–33], $d(CH_2)_5D\text{-}Phe^2Val^4AVP$ [10], $desGly^9d(CH_2)_5Tyr(Et)^2Val^4AVP$ (SK&F 101926) [21], $d(CH_2)_5Tyr(Me)^2Val^4AVP$, $d(CH_2)_5Tyr(Et)^2Val^4AVP$ and $d(CH_2)_5D\text{-}Tyr^2Val^4AVP$ (unpublished results), and the relatively selective $V_2$ receptor antagonists $d(CH_2)_5D\text{-}Phe^2,Ile^4AVP$ [29] and $d(CH_2)_5D\text{-}Ile^2,Val^4AVP$ [28] to rats with effectively identical results. On the other hand, chronic infusion of the selective $V_1$ antagonist, $d(CH_2)_5Tyr(Me)AVP$, produces the anticipated pressor blockade with little or no effect on urine concentrating ability [28, 34, 35].

Several aspects of the responses illustrated in Figs. 1 and 2 are notable. First, the effects occur whether the ADH antagonists are infused (i.p., s.c.) with minipumps or injected (i.v.) once daily for 5 or more days. In vasopressin-replete rats the aquaretic effect is rapid in onset, dose dependent, and transient. The attenuation of the aquaresis in vasopressin-replete rats and the development of antidiuresis in DI rats occur with similar time constants and these effects are not associated with detectable changes in body weight gain, food intake, urinary excretion of

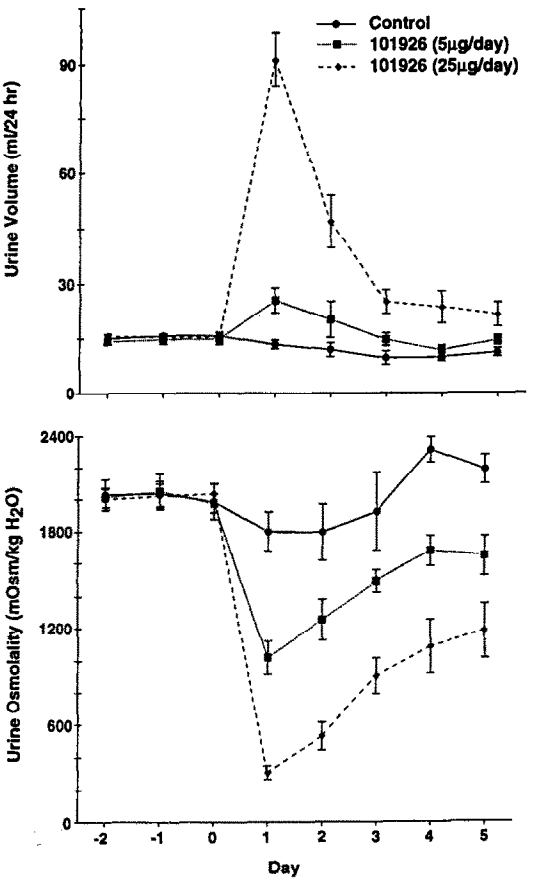


Fig. 1. Dose–response and time–response of the aquaretic effect of SK&F 101926 in vasopressin-replete (Long Evans strain) rats. Rats were housed individually in metabolism cages with free access to food and water. Following establishment of baseline (days −2 through 0) urine volume (mL/day) and urine osmolality (mOsmol/kg $H_2O$), miniature osmotic pumps (Alzet 2001) loaded to deliver 0.9% NaCl (1 μL/hr, control) or SK&F 101926 (5 or 25 μg/day) were inserted subcutaneously, and urine volume and osmolality were monitored daily for days 1 through 5. See Ref. 28 for additional details. Values are means ± SEM, N = 5/group.

solutes, or plasma volume or plasma concentrations of electrolytes ([36], and unpublished observations). Changes in urine flow rate appear to be matched rapidly by changes in water intake ([27], and unpublished observations). In DI rats the antidiuretic effect is slow in onset, compared to the rapid onset of antidiuresis associated with the antidiuretic agonists AVP or dDAVP and the rapid onset of aquaresis associated with the ADH antagonist in vasopressin-replete rats, requiring >24-hr treatment before an effect is detected and up to 5 days of treatment before a maximal effect is achieved. Similarly, recovery of the baseline diuretic condition following drug withdrawal in DI rats is also slow, compared to the rapid recovery and overshoot observed with AVP or dDAVP, requiring days or weeks before the baseline urine flow rates and urine

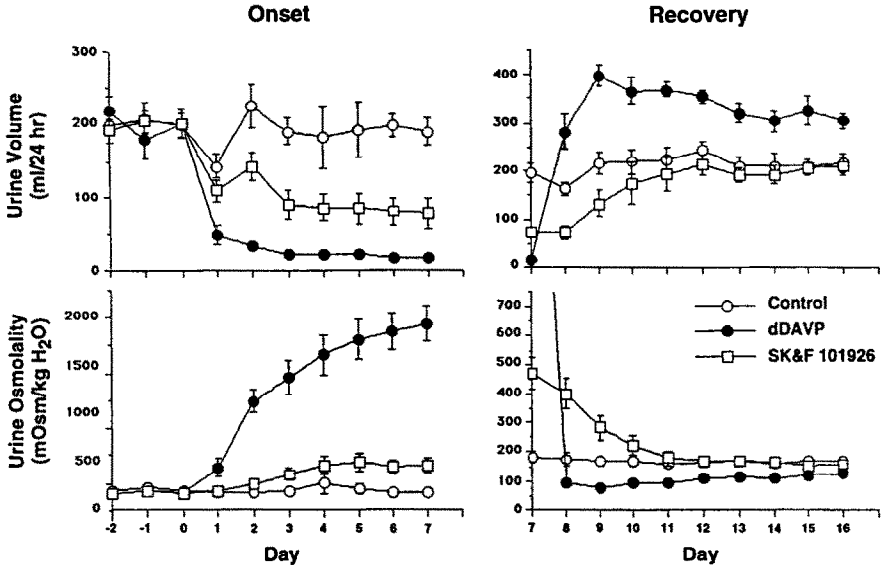


Fig. 2. Comparison of the antidiuretic effects of dDAVP and SK&F 101926 in Brattleboro strain diabetes insipidus rats. Rats were housed individually in metabolism cages with free access to food and water. Following establishment of baseline (days −2 through 0) urine volume (mL/24 hr) and urine osmolality (mOsmol/kg $H_2O$), miniature osmotic pumps (Alzet 2001) loaded to deliver 0.9% NaCl (1 μL/hr, control) dDAVP (2 ng/day), or SK&F 101926 (12 μg/day) were inserted subcutaneously, and urine volume and osmolality were monitored daily for days 1 through 7 (left-hand panels). On day 7 all the pumps were removed, and the rats were monitored daily for an additional 9 days (right-hand panels). See Ref. 28 for additional details. Values are means ± SEM, N = 5/group.

osmolalities are re-established [28, 32, 33, 37]. In DI rats, both the maximal antidiuretic effect and the time to restoration of the baseline diuretic state are dose dependent. Another curious finding is that the antidiuretic state obtained in DI rats after 4 or more days of infusion of an ADH antagonist is apparently insensitive to the acute administration of even supra-maximal doses of vasopressin, dDAVP, or ADH antagonist [28, 33].

Many of the early ADH antagonists had measurable intrinsic ADH activity in the ethanol-anesthetized rat bioassay [16]. Subsequently, ADH antagonists lacking detectable intrinsic ADH activity were identified (desGly$^9$d$(CH_2)_5$D-Tyr(Et)$^2$-Val$^4$AVP; d$(CH_2)_5$D-Phe$^2$Ile$^4$AVP; SK&F 105494) [16, 22, 38–40]; however, several of the latter demonstrated antidiuretic activity when infused in DI rats [28, 29]. The DI rat is a more sensitive model for bioassay of intrinsic ADH activity, and ADH antagonists previously reported to lack intrinsic ADH activity exhibit measurable antidiuretic activity in this model [28, 29]. Table 1 compares the activities of several ADH antagonists given chronically and acutely to rats. All of the compounds are $V_2$ receptor antagonists *in vitro*; all are potent aquaretic agents when given acutely to vasopressin-replete rats; and all are antidiuretic when infused chronically. However, all but one of the compounds exhibit weak ADH activity when bioassayed acutely in DI rats. SK&F 104069 is notable in that it lacked antidiuretic activity when administered acutely in DI rats; indeed, this compound appeared to be diuretic in this model. However, when co-infused chronically with SK&F 101926, SK&F 104069 did not block the antidiuretic activity of SK&F 101926 in DI rats. This observation may be attributed to the lower affinity of SK&F 104069 for $V_2$ receptors, compared to SK&F 101926. It is also notable that the antidiuretic efficacy of ADH antagonists (e.g. the maximal urine concentrating effect observed) given acutely in DI rats is much lower than that observed when the same antagonists are infused for 5 or more days [28, 29]. Hence, the mechanism of antidiuresis of ADH antagonists given acutely appears to be dissociated from the mechanism of antidiuresis of these same compounds given chronically to DI rats.

How are compounds that are potent $V_2$ receptor antagonists *in vitro* and potent ADH antagonists upon single administration to vasopressin-replete rats *in vivo* antidiuretic in DI rats? Those ADH antagonists that have been evaluated for pharmacokinetic parameters in rats have circulating half-lives of approximately 20 min [28]. The metabolism of these antagonists is primarily by cleavage of the linear "tail" portion of the molecule, a route which further reduces the potential for agonist activity [38, 40]. Hence, it is unlikely that ADH antagonists infused into rats either accumulate in plasma or undergo metabolism to form "super" agonists. ADH antagonists given in large doses (>1 mg/kg, i.v.) to rats degranulate mast cells and cause hypotension; however, these effects are subject to a rapid tachyphylaxis and do not occur with repeated dosing and, hence, they cannot contribute to the antidiuretic response discussed here [36]. Gellai and colleagues [42] demonstrated that DI rats

Table 1. Antidiuretic activities of ADH antagonists

| Compound* | ADH activity with chronic infusion in rats† | $K_d$‡ (hog) (nM) | $K_i$‡ (human) (nM) | Aquaretic potency§ ($ED_{300}$, μg/kg) | Urine osmolality‖ (mOsmol/kg $H_2O$) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Pre | Post | Change |
| Control | | | | | 152 ± 9 | 168 ± 10 | 15 ± 8 |
| SK&F 105494 | Agonist | 5.1 ± 1.3 | 3.9 ± 2.7 | 9.3 ± 0.2 | 158 ± 15 | 206 ± 16 | 48 ± 14 |
| SK&F 101926 | Agonist | 11.8 ± 1.2 | 3.6 ± 0.7 | 15.0 ± 2 | 159 ± 7 | 209 ± 15 | 50 ± 14 |
| SK&F 103111 | Agonist | 15.8 ± 6.1 | 4.3 ± 1.0 | 25 ± 5 | 143 ± 5 | 193 ± 23 | 50 ± 22 |
| SK&F 104069 | Agonist | 221 ± 49 | 25.8 | 76.8 ± 3 | 166 ± 14 | 117 ± 8 | −43 ± 7 |
| $d(CH_2)_5Tyr(Me)AVP$ | NA¶ | 369 | 210 | >1000 ± 20 | 167 ± 40 | 271 ± 28 | 104 |

All values are means ± SEM.
* See Refs. 21, 24 and 25 for structures; SK&F 103111 is (4-Me)d$(CH_2)_5$D-Tyr(Et)$^2$Val$^4$AVP.
† Antidiuretic activity observed upon infusion or repeat-daily administration for ≥5 days in rats ([28, 36] and unpublished findings).
‡ See Ref. 15.
§ See Refs. 17 and 41.
‖ All drug doses were 30 μg/kg, given subcutaneously in 0.9% NaCl (1 mL/kg) to DI rats; control DI rats were given 0.9% NaCl (1 mL/kg). Urine was collected for 3 hr; values are means (± SEM) of the 3-hr urine osmolality, N = 5–7/group. See Ref. 28 for additional details.
¶ Not available.

could develop a substantial ADH-independent antidiuresis when subjected to graded dehydration; in those studies body weight was decreased 6–20%. Since no body weight decreases occur during chronic administration of ADH antagonists, dehydration is unlikely to be responsible for the antidiuretic effects in vasopressin-replete or DI rats. Reductions in renal blood flow and glomerular filtration rate (GFR) can also result in antidiuresis in the absence of endogenous vasopressin [43]. $V_1$ receptors are located on glomerular vascular and mesangial components and could mediate reductions in renal hemodynamic parameters [44]. Mah and colleagues [30] investigated whether chronic administration of ADH antagonists was associated with changes in renal hemodynamics in rats; no drug-associated changes were detected. Edwards and colleagues [43] demonstrated antidiuresis in DI rats even in the absence of detectable changes in GFR; however, the urine concentrating ability achieved under these conditions did not approach the concentrating ability achieved by chronic administration of an ADH antagonist. In addition, the antidiuretic activity of ADH antagonists does not appear to require affinity for $V_1$ receptors [28]. Finally, in toxicity studies no histological changes have been observed in the glomeruli or renal vasculature of rats treated with SK&F 101926 or SK&F 105494 [36]. Hence, there is no evidence to support a hemodynamic mechanism of antidiuresis. This conclusion is consistent with other reports that ADH antagonists selectively affect renal epithelial, but not renal vascular functions in healthy animals [45, 46].

The delayed onset and prolonged recovery from the antidiuretic effects of ADH antagonists administered chronically are unusual and are distinguished from the rapid onset and recovery from antidiuretic activity of vasopressin or dDAVP. It has been suggested that this slow onset might be related to a slowly developing medullary concentration gradient in vasopressin-treated DI rats [see Ref. 28]. This mechanism is unlikely as (1) DI rats can rapidly (within 24 hr) produce concentrated urine of ≥1500 mOsmol/kg $H_2O$ when appropriately stimulated with ADH activity [37], and (2) the slowly developing antidiuretic response in DI rats parallels the time course of the loss of the aquaretic response of these compounds in vasopressin-replete rats, in which no medullary concentrating defect is known [29]. On the other hand, a slow antidiuretic response to the same ADH antagonists has been demonstrated in conscious, hydrated, cyclooxygenase-blocked dogs [47], suggesting that the time component may be due to other factors involved in the regulation of the $V_2$ receptor/effector pathway [6].

In isolated tissue or membrane preparations the ADH receptor antagonists discussed in this commentary bind specifically to one population of binding sites, displace bound labeled AVP ([$^3$H]-AVP) with high affinity, and block vasopressin-stimulation of membrane-bound adenylate cyclase and transepithelial water fluxes [11, 15, 18, 19, 48]. Intrinsic agonist activity of ADH antagonists is not detectable in these systems. The effects of vasopressin on the kinetics of renal $V_2$ receptors have been reviewed recently [6, 28]. In the rat, infusion of

vasopressin at rates sufficient to produce plasma vasopressin concentrations comparable to those associated with 48-hr dehydration had no effect on $V_2$ receptor number ($B_{max}$) or binding affinity ($K_d$) [49]. These studies show that autologous down-regulation of $V_2$ receptors is minimal in the normal rat kidney and suggests that basal $V_2$ receptor reserve is relatively low in this species. The effects of ADH antagonists on ADH receptor kinetics in rats are conflicting. Chang and Kimura [50] reported a dose-dependent decrease in [$^3$H]AVP binding and vasopressin-dependent adenylate cyclase activity in rats given increasing doses of d(CH$_2$)$_5$D-Tyr(Et)$^2$Val$^4$AVP 60-min prior to being killed. Similarly, Mah and colleagues [48] reported undetectable [$^3$H]AVP binding in DI rats during infusion of 72 μg/day of d(CH$_2$)$_5$D-Tyr(Et)$^2$-Val$^4$AVP; in this study [$^3$H]AVP binding had not returned to pretreatment levels after 6 days post-treatment. We also measured a reduction in [$^3$H]-AVP binding in DI rats during infusion of a smaller dose (10 μg/day) of desGly$^9$d(CH$_2$)$_5$D-Tyr(Et)$^2$Val$^4$AVP; however, at 24 and 48 hr post-infusion we measured [$^3$H]AVP binding at levels significantly above baseline values ([28], and unreported results). We and others have observed that ADH antagonist/receptor complexes may dissociate much more slowly than ADH/receptor complexes ([48], and unpublished observations). We interpret the decreases in [$^3$H]AVP binding measured in rats injected acutely or infused chronically with ADH antagonists as masking of $V_2$ receptors by the ADH antagonist. However, masking cannot account for the elevated levels of [$^3$H]AVP binding that we observed at ≥24-hr post-infusion. We conclude, in contrast to others [48, 50], that infusion of ADH antagonists in DI rats leads to up-regulation of ADH receptors and sensitization of the kidney to the weak antidiuretic activities of ADH antagonists and/or other circulating peptides (e.g. oxytocin) [28]. We speculate that similar up-regulation occurs in ADH antagonist-infused vasopressin-replete rats.

Up-regulation of $V_2$ receptors could account for all of the observations made in ADH antagonist-infused vasopressin-replete and DI rats and could provide explanations for the conflicting *in vitro* and *in vivo* findings. Up-regulation of $V_2$ receptors (increasing receptor reserve) could increase the sensitivity of the target cells to low-level intrinsic ADH activity of ADH antagonists and account for the antidiuretic response observed in DI rats and the loss of the aquaretic response in ADH-replete rats [see Ref. 6]. Up-regulation of $V_2$ receptors would explain the apparent loss of responsiveness of the rat kidney to acute administration of even supra-maximal doses of ADH agonists or antagonists [28, 33]. The slow time course of the up-regulation and subsequent down-regulation responses could account for the slow onset and prolonged duration of the antidiuretic response in DI rats and offers an explanation for the parallel response times in ADH-replete and DI rats infused with ADH antagonists.

The molecular mechanism of ADH antagonist-induced $V_2$ receptor up-regulation is unknown. Vasopressin/receptor complexes are internalized in a process that may play a role in desensitization of the target cells by reducing the number of cell surface receptors [5, 51]. It has been shown recently that an ADH antagonist/receptor complex is not internalized [52]. If internalization of the agonist/receptor complex is responsible for receptor down-regulation, then failure of ADH antagonist/receptor complexes to undergo endocytosis would cause the surface receptor populations of target cells to increase. We surmise that under conditions of continuous exposure to ADH antagonist most if not all renal $V_2$ surface receptors are ADH antagonist/receptor complexes.

Are the antidiuretic activity and $V_2$ receptor up-regulation associated with ADH antagonists intrinsic properties of high affinity peptide ADH antagonists (e.g. a "drug class" effect), or are they intrinsic properties of the renal $V_2$ receptor/effector pathway? Agonist activity has previously limited the clinical utility of another class of peptides, the peptide antagonists of angiotensin II receptors (e.g. saralasin), but has apparently not been detected with the non-peptide angiotensin II receptor antagonists (e.g. losartan) [53, 54]. Hence, there is precedent to suggest that new classes of non-peptide ADH receptor antagonists may have a greatly reduced potential for intrinsic ADH activity [55, 56]. However, whether or not these compounds will be associated with the similar activity profiles as shown in Figs. 1 and 2, cannot be predicted as these may depend upon the cellular processing of non-peptide ADH antagonist/$V_2$ receptor complexes and the resulting effects on surface receptor densities.

Finally, the relationships (if any) between the antidiuretic effect of ADH antagonists in rats and the antidiuretic effects of these compounds in other species, including humans, are unknown. SK&F 101926 induced up-regulation of $V_2$ receptors and antidiuresis in rats takes several days of continuous exposure to develop fully [28]. However, the sensitivity of the dog kidney to the intrinsic agonist and antagonist activities of SK&F 101926 changes much more rapidly [6]. We speculate that inter-species differences in basal set-points and time-constants of the molecular mechanisms regulating the sensitivity of the renal $V_2$ receptor/effector pathway may ultimately explain the paradoxical responses to SK&F 101926 and SK&F 105494 and other ADH antagonists.

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