VPA-985, A NONPEPTIDE ORALLY ACTIVE AND SELECTIVE VASOPRESSIN V2 RECEPTOR ANTAGONIST

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INTRODUCTION

The introduction of the thiazides as orally-active diuretics about forty years ago (1), and other more effective low-ceiling diuretics thereafter, revolutionized the treatment of edema, ascites, hypertension and related diseases. Later, the addition of potent high-ceiling (loop) and potassium-sparing diuretics provided clinicians with a wide choice of diuretics to eliminate retained sodium and water (2). However, it was soon evident that many patients became refractory to these saluretic agents and some developed hyponatremia (serum sodium $< 130$ mEq/L) (3, 4, 5). Hyponatremia also occurs in the syndrome of inappropriate antidiuretic hormone secretion (SIADH), in patients with congestive heart failure (CHF), liver cirrhosis with ascites, renal failure, and many other disorders where the plasma vasopressin concentrations are inappropriately high for any given plasma osmolality. Under these conditions, an aquaretic (water diuretic), not a conventional diuretic, would be the drug of choice to promote the excretion of the retained body water and to normalize plasma osmolality and sodium concentration (6, 7, 8). As vasopressin (AVP, antidiuretic hormone (ADH)) acting at $V_2$ receptors in the collecting ducts controls water re-absorption (7, 8), considerable effort has been spent over many years to develop vasopressin $V_2$ receptor antagonists or agents that could inhibit the release of vasopressin from the posterior pituitary (8,9). Many peptide vasopressin analogs have been developed as vasopressin $V_2$ receptor antagonists, and two of them, SK&F 101926 and SK&F 105494, showed excellent $V_2$ antagonistic activity in many animal models, including non-

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human primates. Unfortunately, in humans, both compounds behaved as vasopressin \( V_2 \) agonists (9). Recently, three nonpeptidic and orally active vasopressin receptor antagonists have been described in the literature. The first two, OPC-31260 (10), and SR 121463A (11), are \( V_2 \) selective, while the third compound, YM087 (12), is a dual \( V_{1a}/V_2 \) receptor antagonist.

We report here the preclinical pharmacology of VPA-985 (5-Fluoro-2-methyl-N-[4-(5H-pyrrolo[2,1-c][1,4]benzodiazepin-10(11H)-yl carbonyl)-3 chlorophenyl] benzamide) a potent, selective, orally-active, nonpeptide vasopressin \( V_2 \) receptor antagonist discovered at Wyeth-Ayerst Research (13). VPA-985 (WAY-VPA-985) has already been studied in patients with congestive heart failure where it has shown a potent aquaretic effect (14).

RESULTS

Effects of VPA-985 on Receptor Binding and Cyclic AMP Production

VPA-985 inhibited the binding of \( [\text{H}] \)-AVP to native \( V_2 \) receptors in membranes isolated from rat and dog renal medulla with \( K_i \) values of 0.48 ± 0.03 and 0.82 ± 0.03 nM (SEM), respectively. VPA-985 also inhibited \( [\text{H}] \)-AVP binding in membranes of cultured murine fibroblasts (LV2 cell line) expressing the human \( V_2 \) receptor with a \( K_i \) of 0.60 ±0.02 nM. Scatchard analysis of binding to cloned human \( V_2 \) receptors showed that the dissociation constant (\( K_d \)) of \( [\text{H}] \)-AVP increased from 0.79±0.03 nM in the vehicle-control to 1.04 nM in the presence of 0.5 nM VPA-985, while the maximum binding (\( B_{\text{max}} \)) remained unchanged (1.14±0.08 vs. 1.02 fmole/\( \mu \)g protein, respectively). These results suggest that VPA-985 is a competitive inhibitor of AVP binding to \( V_2 \) receptors.

Cyclic-AMP is the second messenger of AVP at the \( V_2 \) receptors. AVP stimulated cAMP production in LV2 cells expressing human \( V_2 \) receptors with maximum effect at 1 nM and \( EC_{50} = 53.3 \pm 4.4 \) pM. VPA-985 (up to 10 \( \mu \)M) had no effect on basal cAMP generation, but inhibited the AVP-induced cAMP generation (\( IC_{50} = 6 \pm 1 \) nM against 1 nM AVP stimulation). In addition, VPA-985 (1.0 \( \mu \)M) completely inhibited the rise in cAMP induced by 0.05 \( \mu \)M AVP in membranes prepared from the rat kidney medulla.

VPA-985 was further characterized in vasopressin \( V_{1a} \) and oxytocin receptor binding assays. VPA-985 inhibited the binding of the Manning compound (d(CH\(_2\))\(_4\),Tyr (Me\(_i\),Arg 8)-vasopressin), a \( V_{1a} \)-selective antagonist, to \( V_{1a} \) receptors in membranes from rat hepatocytes and human platelets with \( K_i \) values of 82 ± 15 nM and 55 ± 12 nM, respectively. VPA-985 also inhibited the binding of the Manning compound to membranes from CHO cells expressing human \( V_{1a} \) receptors with a \( K_i = 74 \pm 3 \) nM. In comparison, AVP and two \( V_{1a} \)-selective peptide analogs (Manning compound and Phenylac\(_1\),D-Tyr(Me)\(_2\),Arg\(_{6,8}\)-Lys-NH\(_2\)\(_9\)-AVP) inhibited the binding of \( [\text{H}] \)-Manning compound with \( K_i \) values of 0.30±0.01, 0.86±0.03 and 0.14±0.01 nM, respectively. VPA-985 showed some affinity for the oxytocin receptor and inhibited the binding of \( [\text{H}] \)Oxytocin to native receptors in membranes isolated from rat uterus with a \( K_i \) of 39 ± 6 nM and to human oxytocin receptors expressed in CHO cells with \( K_i = 275 \pm 23 \) nM.

AVP induces proliferation of NIH-3T3 cells transfected with human \( V_{1b} \) receptors. VPA-985 had no effect on basal or AVP-induced cell proliferation, suggesting that VPA-985 had no activity at the \( V_{1b} \) receptor.

These receptor binding and cAMP production studies show that VPA-985 (i) is a potent competitive AVP \( V_2 \) receptor antagonist with no partial agonist activity, (ii) is approxi-