0026-895X/87/030267-06$02.00/0

MOLECULAR PHARMACOLOGY, 31: 267-272

A Novel Radiolabeled Vasopressin Antagonist: [^{3}H-Phe]-desGlyd(CH$_2$)$_5$D-Tyr(Et)VAVP, [^{3}H]-SK&F 101926

F. L. STASSEN, G. HECKMAN, D. SCHMIDT, P. NAMBI, N. AIYAR, S. LANDVATTER, and S. T. CROOKE

Department of Molecular Pharmacology, Smith Kline and French Laboratories, P. O. Box 7929, Philadelphia, Pennsylvania 19101

Received August 7, 1986; Accepted December 10, 1986

SUMMARY

We report the vasopressin receptor-binding properties of [^{3}H-Phe]-desGlyd(CH$_2$)$_5$D-Tyr(Et)VAVP, [^{3}H]-SK&F 101926, the first radiolabeled vasopressin receptor antagonist. We chose to radiolabel SK&F 101926 because this vasopressin analog is a potent antagonist of vascular V_1 and renal V_2 vasopressin receptors in all species studied. [^{3}H]-SK&F 101926 bound with a single high affinity to intact vascular smooth muscle cells (A-10; K_D = 0.5 nM), and plasma membranes of A-10 cells (K_D = 0.4 nM) and rat liver (K_D = 0.2 nM). In competition experiments with [^{3}H]-SK&F 101926 and [^{3}H]arginine vasopressin ([^{3}H]AVP) using cell and liver membranes, the affinity rank orders of vasopressin analogs were the same and were typical for the V_1 receptor subtype. In competition binding experiments with [^{3}H]-SK&F 101926 using cell and liver membranes, guanosine 5′-(β,γ-imido)triphosphate did not significantly alter the affinity of the V_1 antagonist d(CH$_2$)$_5$Tyr(Me)AVP, but the affinity of AVP was decreased. These data indicate that the V_1 receptor can exist in at least two affinity states that are modulated by guanine nucleotides. [^{3}H]-SK&F 101926 also bound specifically and with high affinity to V_2 receptors of MDCK cells. We conclude that [^{3}H]-SK&F 101926 binds with high affinity to V_1 and V_2 vasopressin receptors and is a powerful new tool for the identification of vasopressin receptors and the study of molecular mechanisms involved in the interaction of vasopressin with its receptors.

Direct identification and characterization of vasopressin receptors has been performed with the radiolabeled vasopressin agonists, [^{3}H]lysine vasopressin, [^{3}H]AVP, and [3-^{125}I-Tyr]AVP. Radiolabeled antagonists are more suitable ligands to quantify and characterize hormone receptors than agonists, because agonists can bind to one class of receptors with different affinities depending on the receptor state [reviewed by Lefkowitz *et al.* (1)], whereas antagonists bind with only one affinity, regardless of the receptor state. We have recently presented evidence that vasopressin receptors of the vascular V_1 subtype can exist in two affinity states (2). Therefore, a radiolabeled antagonist would be a useful tool to study vasopressin receptors.

We have chosen desGlyd(CH$_2$)$_5$D-Tyr(Et)VAVP for radiolabeling, because this vasopressin analog is a potent antagonist at all vasopressin receptors studied. desGlyd(CH$_2$)$_5$D-Tyr(Et)VAVP (a) binds with high affinity to hepatic V_1 receptors (3); (b) is a potent antagonist of vasopressin-induced increase in blood pressure and contraction of rat aortic rings (3) and human mesenteric arteries (3a); (c) binds with high affinity to pig kidney receptors; (d) is a potent inhibitor of vasopressin-induced activation of adenylate cyclase of medullary membranes of pig, rat, squirrel monkey, and human kidney; and (e) is a potent aquaretic agent in rat, squirrel monkey, and dog (3, 4).

Here we report the vasopressin receptor-binding properties of the radiolabeled vasopressin antagonist, [^{3}H-Phe]-desGlyd(CH$_2$)$_5$D-Tyr(Et)VAVP, [^{3}H]-SK&F 101926. We show that [^{3}H]-SK&F 101926 bound specifically and with high affinity to V_1 receptors of vascular smooth muscle cells and liver and to renal V_2 vasopressin receptors. In addition, we demonstrate that Gpp(NH)p decreased the affinity of V_1 receptors for vasopressin agonists but not for antagonists.

Experimental Procedures

Materials. [Phenylalanyl-3,4,5-3H(N)]desGlyd(CH$_2$)$_5$D-Tyr(Et)-VAVP, [^{3}H]-SK&F 101926 (specific activity 37.9 Ci/mmol), was pre-

ABBREVIATIONS: AVP, arginine vasopressin; desGlyd(CH$_2$)$_5$D-Tyr(Et)VAVP or SK&F 101926, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-D-(*O*-ethyl)tyrosine, 4-valine, 8-arginine, 9-desglycine]vasopressin; Gpp(NH)p, guanosine 5′-(β,γ-imido)triphosphate; dDAVP, [1-desamino,8-D-arginine]vasopressin; d(CH$_2$)$_5$Tyr(Me)AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-(*O*-methyl)tyrosine, 8-arginine]vasopressin; d(CH$_2$)$_5$Tyr(Et)VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-(*O*-ethyl)tyrosine, 4-valine, 8-arginine]vasopressin; d(CH$_2$)$_5$D-Ile-VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-D-isoleucine, 4-valine, 8-arginine]-vasopressin; d(CH$_2$)$_5$D-Tyr(Et)VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-D-(*O*-ethyl)tyrosine, 4-valine, 8-arginine]vasopressin; EDTA, ethylenediaminetetraacetate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DPBS^{2+}, Dulbecco's phosphate-buffered saline with 10 mM $MgCl_2$, 0.7 mM $CaCl_2$, 0.1% glucose, and 0.2% bovine serum albumin; BSA, bovine serum albumin.

pared at SK&F Laboratories (Philadelphia, PA). The radioligand was prepared by solid phase peptide synthesis. Phenylalanine was incorporated into the peptide sequence as *t*-Boc-phenylalanine, [2,6-^{3}H(N)] (New England Nuclear, Boston, MA). The radiochemical purity was greater than 97%. [^{3}H]AVP (40 Ci/mmol) was purchased from New England Nuclear; AVP and dDAVP were from Bachem (Torrance, CA). Other vasopressin analogs used were: desGlyd$(CH_2)_5$D-Tyr(Et)VAVP (SK&F 101926), d$(CH_2)_5$Tyr(Me)AVP, d$(CH_2)_5$-Tyr(Et)VAVP, d$(CH_2)_5$D-IleVAVP, and d$(CH_2)_5$D-Tyr(Et)VAVP. These analogs were synthesized at SK&F Laboratories.

Fetal calf serum was obtained from KC Biologicals (Lanexa, KS); Dulbecco's Modified Eagle's medium, Dulbecco's phosphate-buffered saline (0.5 mM $MgCl_2$, 0.7 mM $CaCl_2$), and trypsin were from Gibco (Grand Island, NY); and bradykinin, atriopeptin II, somatostatin, tyrosyl somatostatin, and BSA (fraction V) were from Sigma Chemical Co. (St. Louis, MO). Angiotensin II was from Calbiochem (La Jolla, CA).

Cell culture. Vascular smooth muscle cells (A-10) were cultured in Dulbecco's modified Eagle's medium plus 20% fetal calf serum. Before initiating the experiments, the cells were subcloned twice by limiting dilution. All experiments were performed with cells passaged once a week for no more than 4 months. The cells were removed from the flasks by incubating with 0.25% trypsin containing 0.25% EDTA (pH 7.5). Culture wells (35 mm diameter; six-well Linbro plates) were seeded with 1 ml of medium containing 75,000 cells. Experiments were performed after 3 days in culture. The dog kidney cells (MDCK) were cultured as described for the A-10 cells. Experiments were performed after 4 days in culture.

Preparation of plasma membranes. Plasma membranes of A-10 cells were prepared as previously described (2). The membrane pellet was suspended in 5 mM Tris·HCl, pH 7.5 (at 30°), containing 3 mM $MgCl_2$ and 1 mM EDTA (from 0.5 M EDTA, pH set at 7.3 with Tris base at 20°; hypotonic Tris buffer). The membrane suspension was used immediately.

Plasma membranes of liver of male Sprague-Dawley rats (225–275 g) were prepared according to the procedure of Neville (5) up to step 11. The membranes were suspended in hypotonic Tris buffer and stored frozen in liquid nitrogen.

[^{3}H]-SK&F 101926 binding to cell and liver membranes. [^{3}H]-SK&F 101926 binding to cell and liver membranes was performed in a mixture (final volume 500 μl) containing 10 mM HEPES buffer, pH 7.4, 10 mM $MgCl_2$, 0.1% BSA, 50–100 μg of cell membrane protein, and [^{3}H]-SK&F 101926. Nonspecific binding was determined with 10 μM AVP. The incubation was carried out for 90 min at 37° unless indicated otherwise. Bound and free [^{3}H]-SK&F 101926 were separated by centrifugation at 12,000 × *g* for 5 min. The membrane pellet was treated with NCS tissue solubilizer (Amersham, Arlington Heights, IL) and the radioactivity was measured. The affinity of unlabeled ligands for vasopressin receptors was determined in competitive binding experiments using 0.2 nM [^{3}H]-SK&F 101926 or [^{3}H]AVP (1.5 nM and 5 nM for liver and cell membranes, respectively). The affinities were expressed as IC_{50} values, i.e., the concentration required for 50% inhibition of specific binding.

[^{3}H]-SK&F 101926 binding to A-10 and MDCK cells in monolayer. The culture medium was removed, the cells were washed with $DPBS^{2+}$ ($DPBS^{2+}$ with 10 mM $MgCl_2$, 0.7 mM $CaCl_2$, 0.1% glucose, 0.2% BSA), and the binding was initiated with 1 ml of $DPBS^{2+}$ containing [^{3}H]-SK&F 101926. Nonspecific binding was determined in the presence of 10 μM AVP. At the end of the incubation period the medium was removed and the cells were washed with 1 ml of ice-cold $DPBS^{2+}$. The cells were scraped in ice-cold $DPBS^{2+}$, washed rapidly on Amicon filters (0.45 μm), and the cell-associated radioactivity was measured.

Dissociation of [^{3}H]-SK&F 101926 from cell membranes and intact cells. Binding of [^{3}H]-SK&F 101926 (1.0 nM) to cell membranes was performed at 37° as described above. After 90 min, the incubation mixture was diluted with 1 volume of incubation mixture containing 20 μM AVP without radioligand and membranes. After different times at 37°, samples were withdrawn and membrane-bound [^{3}H]-SK&F 101926 was determined. At each time point specifically bound radioligand was calculated by subtracting radioligand bound in the presence of 10 μM AVP.

[^{3}H]-SK&F 101926 (1.8 nM) was incubated with cells in monolayer with or without 10 μM AVP for 10 min at 37°, the medium was removed, the monolayer was washed twice with $DPBS^{2+}$, and [^{3}H]-SK&F 101926 was allowed to dissociate in $DPBS^{2+}$ at 37° with or without 10 μM AVP or 10 μM SK&F 101926 for the times indicated. Subsequently, $DPBS^{2+}$ was removed and specifically bound [^{3}H]-SK&F 101926 was determined as described above.

Protein determination. Protein was measured with the Folin reagent (6) and BSA was the standard.

Statistical evaluation. Experimental values are reported as the mean ± standard error. The saturation equilibrium binding data to cell membranes were analyzed by a nonlinear least squares curve-fitting procedure using a generalized model for complex ligand-receptor systems (Scatfit) (7). The calculated K_D and B_{max} values are presented with the standard error of the estimate.

Results

[^{3}H]-SK&F 101926 bound specifically and with high affinity to membranes of vascular smooth muscle cells (A-10). Fig. 1 shows binding of [^{3}H]-SK&F 101926 to cell membranes as a function of time. Equilibrium binding at 37° was reached after

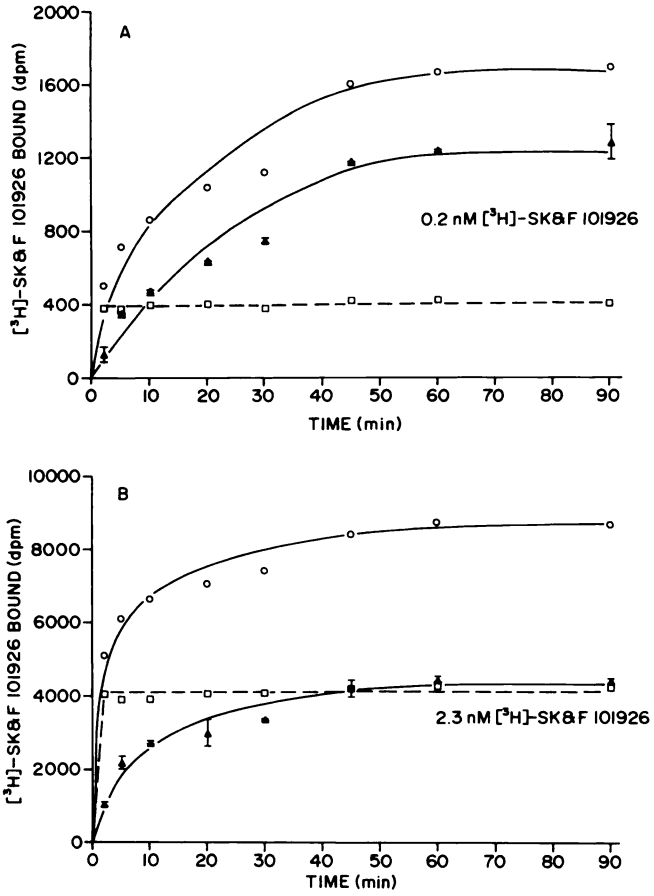

Fig. 1. [^{3}H]-SK&F 101926 binding to membranes of A-10 cells as a function of time. Cell membranes (49.8 μg of protein) were incubated with 0.2 nM [^{3}H]-SK&F 101926 (A) or 2.3 nM [^{3}H]-SK&F 101926 (B) at 37° with (□) or without (○) 10 μM AVP. Mean values of triplicate determinations are presented. Standard error values for specific binding (Δ) are given.

45 and 60 min with 2.3 nM and 0.2 nM [3H]-SK&F 101926, respectively. Maximum nonspecific binding in the presence of 10 μM AVP was reached after 2 min, the shortest time used. Nonspecific binding reached 25 and 50% of total binding at 0.2 nM and 2.3 nM [3H]-SK&F 101926, respectively.

Specific binding of [3H]-SK&F 101926 to cell membranes was reversible (Fig. 2). The cell membranes were incubated with 1.0 nM [3H]-SK&F 101926 with and without 10 μM AVP for 90 min at 37°. Then 1 volume of incubation mixture with 20 μM AVP but without [3H]-SK&F 101926 and membranes was added. [3H]-SK&F 101926 binding was determined after continued incubation at 37° for the times indicated. After 60 min, specifically bound [3H]-SK&F 101926 was almost completely dissociated ($t_{1/2}$ was about 15 min).

Specific binding of [3H]-SK&F 101926 to cell membranes was saturable (Fig. 3). Cell membranes were incubated with [3H]-SK&F 101926 at concentrations from 0.2 nM to 7.0 nM for 90 min at 37°. Nonspecific binding was determined in the presence of 10 μM AVP. Saturation was reached at 3 nM [3H]-SK&F 101926. Scatchard analysis indicated a linear fit (r = 0.90), suggesting the presence of a single high affinity binding site. One-site fitting of the data employing Scatfit resulted in a $K_D = 0.4 \pm 0.2$ nM and a $B_{max} = 95 \pm 21$ fmol/10^6 cells. The number of binding sites correlated well with the number of high affinity vasopressin-binding sites previously determined with [3H] AVP at 37° using A-10 cells in monolayer and cell membranes (2).

[3H]-SK&F 101926 binding to cell membranes was competitively inhibited by AVP and the vasopressin analogs d(CH2)5Tyr(Me)AVP, a V_1-selective antagonist (8), d(CH2)5Tyr(Et)VAVP, a mixed V_1/V_2 antagonist (9), d(CH2)5D-IleVAVP, a V_2-selective antagonist (10), and dDAVP, a selective V_2 agonist (Fig. 4). The most potent inhibitor was the selective V_1 antagonist d(CH2)5Tyr(Me)AVP; the least potent was the selective V_2 agonist dDAVP. [3H]-SK&F

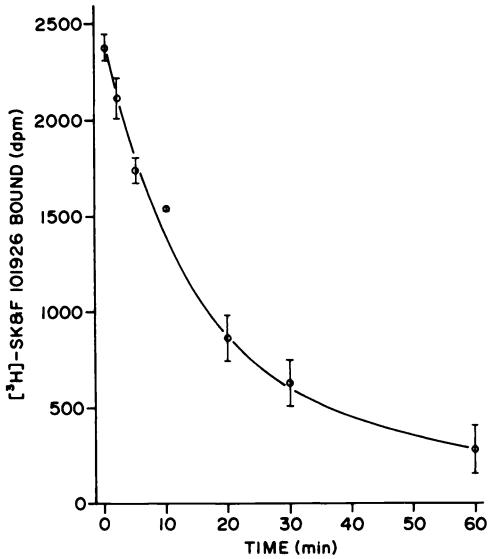

Fig. 2. Dissociation of [3H]-SK&F 101926 from membranes of A-10 cells. Cell membranes were incubated with 1.0 nM [3H]-SK&F 101926 at 37° with or without 10 μM AVP. After 90 min, the incubation mixtures were diluted 2-fold with incubation mixture without cell membranes and [3H]-SK&F 101926, with 20 μM AVP. The incubation was continued and bound [3H]-SK&F 101926 was determined at the times indicated. Mean values ± standard errors of triplicate determinations are presented. The experiment was repeated with similar results.

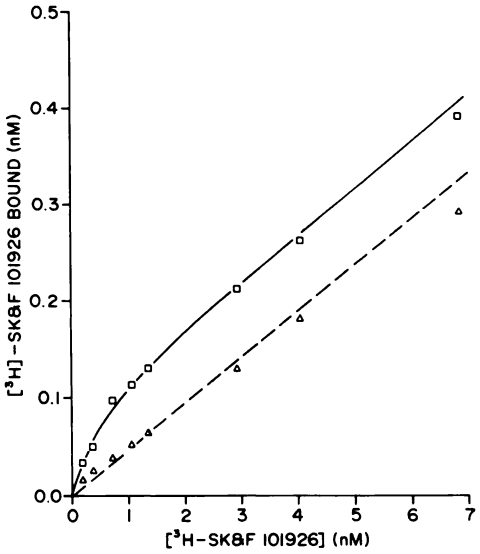

Fig. 3. Saturation equilibrium binding of [3H]-SK&F 101926 to membranes of A-10 cells. Cell membranes (62 μg of protein) were incubated with increasing concentrations of [3H]-SK&F 101926 for 90 min at 37° with (Δ) or without (□) 10 μM AVP. Mean values of triplicate determinations are presented. The individual values did not differ from the mean by more than 5%.

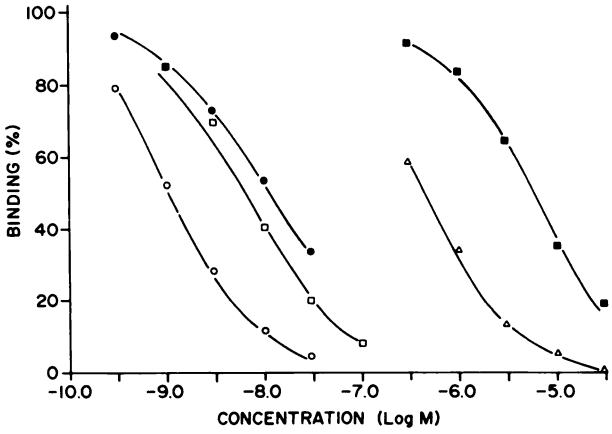

Fig. 4. Inhibition of [3H]-SK&F 101926 binding to membranes of A-10 cells. Cell membranes (50.6 μg of protein) were incubated for 90 min at 37° with 0.2 nM [3H]-SK&F 101926 in the presence of AVP (●), dDAVP (■), d(CH2)5Tyr(Me)AVP (○), d(CH2)5Tyr(Et)VAVP (□) or d(CH2)5D-Ile-VAVP (Δ). Nonspecific binding was determined in the presence of 10 μM AVP. Mean values of triplicate determinations are presented. The individual values did not differ from the mean by more than 10%.

101926 binding to cell membranes was not affected by bradykinin, angiotensin II (10^{-4} and 10^{-5} M), atriopeptin II, somatostatin, or Tyr-somatostatin (10^{-6} and 10^{-7} M; data not shown).

[3H]-SK&F 101926 also bound to rat liver membranes in a specific and saturable fashion with one high affinity (Fig. 5; K_D = 0.2 nM, B_{max} = 2.4 pmol/mg of protein). Nonspecific binding was about 25% at 0.2 nM [3H]-SK&F 101926. The binding was competitively inhibited by the vasopressin analogs with the same affinity rank order as that observed with the A-10 cell membranes (Table 1). The affinity rank order obtained with [3H]-SK&F 101926 was also the same as that obtained with [3H]AVP (Table 1). These data indicate that [3H]-SK&F 101926 bound with high affinity to the V_1 vasopressin receptors of smooth muscle cells and rat liver.

The effects of Gpp(NH)p on the inhibition of binding of

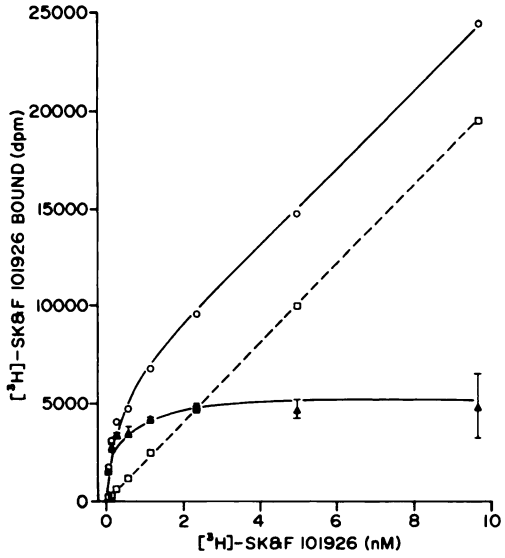

Fig. 5. Saturation equilibrium binding of [³H]-SK&F 101926 to rat liver membranes. Rat liver membranes (25 μg of protein) were incubated with increasing concentrations of [³H]-SK&F 101926 for 90 min at 37° with (□) or without 10 μM AVP (○). Mean values of triplicate determinations are presented. Standard error values for specific binding (Δ) are given. The experiment was repeated with similar results.

TABLE 1

Comparison of affinity rank orders of vasopressin analogs as determined with [³H]-SK&F 101926 and [³H]AVP in membranes of rat liver and smooth muscle cells

Membranes of rat liver (25 μg of protein) and A-10 cells (50.6 μg of protein) were incubated with 0.2 nM [³H]-SK&F 101926 or [³H]AVP at 1.5 nM (footnote *a*) or 5 nM (footnote *b*) for 90 min at 37° with increasing concentrations of the analogs. Nonspecific binding of both radioligands was determined with 10 μM AVP. Mean values of triplicate determinations are presented.

Analog structure	Rat Liver (IC_{50})		A-10 Cells (IC_{50})	
	[³H]-SK&F 101926	[³H]AVP[a]	[³H]-SK&F 101926	[³H]AVP[b]
	nM		nM	
d(CH_2)$_5$Tyr(Me)AVP	1.7	2.5	1.3	10.0
d(CH_2)$_5$Tyr(Et)VAVP	10.2	36.6	7.3	93.0
d(CH_2)$_5$D-Ile VAVP	673.0	1202.0	347.0	3300.0
AVP	16.7	3.0	11.8	8.2
dDAVP	6000.0	742.0	5130.0	6200.0

[³H]-SK&F 101926 to cell membranes by AVP and the antagonist d(CH_2)$_5$Tyr(Me)AVP are shown in Fig. 6. Gpp(NH)p did not significantly affect the competition curve of d(CH_2)$_5$Tyr(Me)AVP, but the competition curve of AVP was shifted to the right, indicating decreased affinity of the receptors for agonists. Similar data were obtained with rat liver membranes (data not shown). These data support our previous contention that guanine nucleotides are involved in the regulation of the affinity of V_1 vasopressin receptors (3, 11).

Specific, saturable binding was also observed when [³H]-SK&F 101926 was incubated for 20 min at 37° with cells in monolayer (Fig. 7). The Scatchard plot was linear (not shown). The K_D = 0.5 ± 0.1 nM and B_{max} = 86 ± 5 fmol/10⁶ cells (n = 3). These values were similar to those for binding to plasma membranes of the A-10 cells. The specific binding sites probably represent vascular vasopressin receptors because the most potent inhibitor of [³H]-SK&F 101926 binding was the V_1-selective antagonist d(CH_2)$_5$Tyr(Me)AVP and the least potent were the V_2-selective antagonist d(CH_2)$_5$D-IleVAVP and the V_2-selective agonist dDAVP (data not shown).

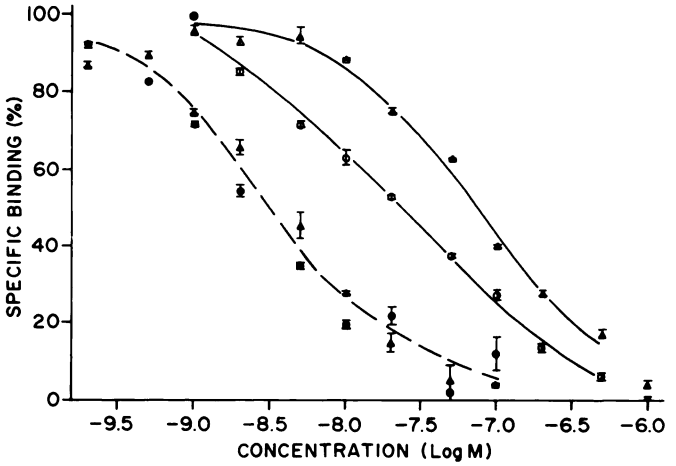

Fig. 6. Effect of Gpp(NH)p on the inhibition of [³H]-SK&F 101926 binding to membranes of A-10 cells by AVP and d(CH_2)$_5$Tyr(Me)AVP. Cell membranes (166 μg of protein) were incubated for 90 min at 37° with 2.0 nM [³H]-SK&F 101926 with AVP (○, Δ) or d(CH_2)$_5$Tyr(Me)AVP (●, ▲) at the concentrations indicated with (Δ, ▲) or without (○, ●) 100 μM Gpp(NH)p. AVP (10 μM) was used to determine nonspecific binding. Mean values ± standard errors of triplicate determinations are presented. The experiment was repeated with similar results.

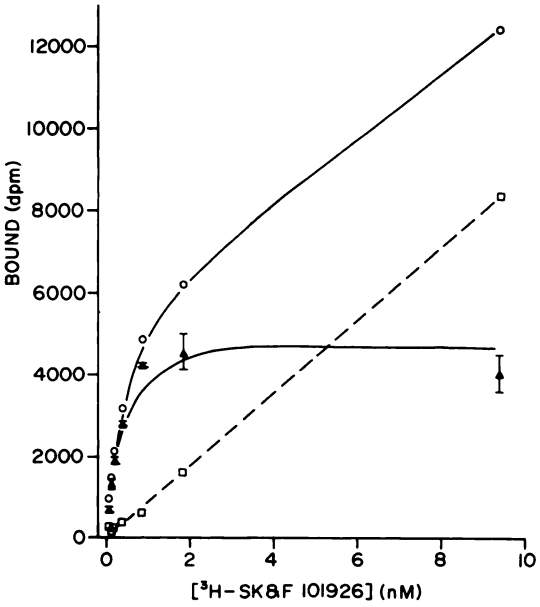

Fig. 7. [³H]-SK&F 101926 binding to A-10 cells in monolayer. [³H]-SK&F 101926 in DPBS^{2+} was incubated with cells in monolayer for 20 min at 37° with (□) or without (○) 10 μM AVP. Mean values of triplicate determinations are presented. Standard error values of specific binding (Δ) are given. The experiment was repeated three times with similar results.

Specifically bound [³H]-SK&F 101926 rapidly dissociated from intact cells with a $t_{1/2}$ of about 10 min (Fig. 8). The dissociation in the presence of excess AVP and SK&F 101926 was complete after 60 min. However, in the absence of excess AVP or SK&F 101926, after 60 min 52% of [³H]-SK&F 101926 remained associated with the cells. These data might suggest negative cooperativity between vasopressin receptors at high concentrations of ligand.

[³H]-SK&F 101926 also bound to MDCK cells in monolayer. The binding was specific and saturable (Fig. 9). The Scatchard plot indicated a K_D = 0.5 nM and a B_{max} = 5.2 fmol/10⁶ cells. In competition binding experiments the selective V_1 antagonist

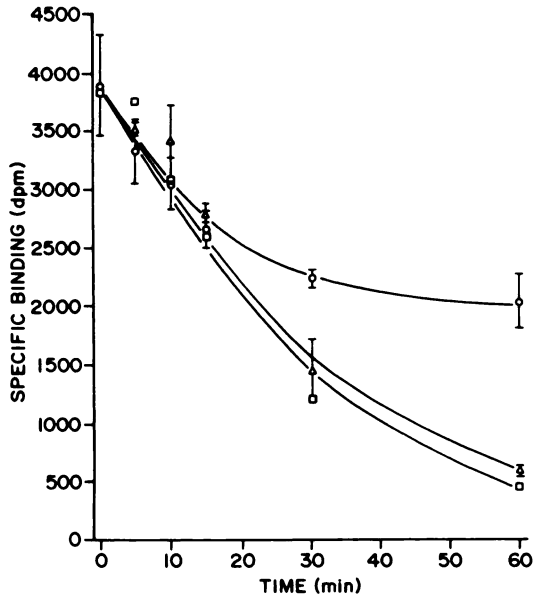

Fig. 8. Dissociation of [³H]-SK&F 101926 from A-10 cells in monolayer. The cells were incubated with 1.8 nM [³H]-SK&F 101926 in $DPBS^{2+}$ for 10 min at 37°. The medium was removed, the cells were washed twice with $DPBS^{2+}$, and [³H]-SK&F 101926 was allowed to dissociate in $DPBS^{2+}$ with (□) or without (○) 10 μM AVP, or with 10 μM SK&F 101926 (△) for the times indicated. Bound [³H]-SK&F 101926 was determined. Nonspecific binding in the presence of 10 μM AVP was subtracted at each time. Mean values ± standard errors are presented. The experiment was repeated twice with similar results.

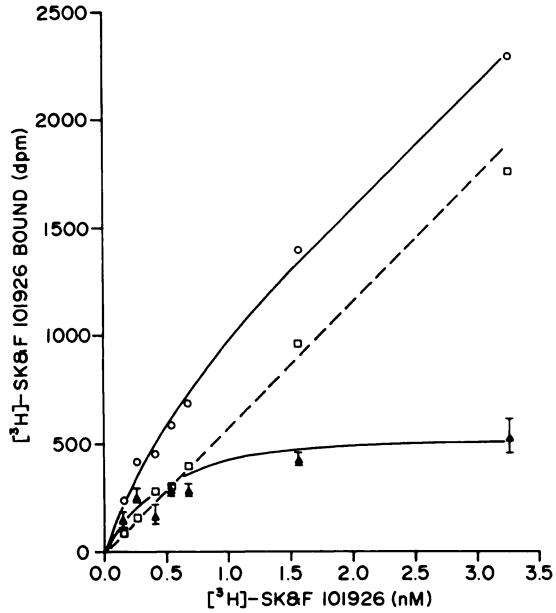

Fig. 9. [³H]-SK&F 101926 binding to MDCK cells in monolayer. [³H]-SK&F 101926 in $DPBS^{2+}$ was incubated with the cells in monolayer for 20 min at 37° with (□) or without (○) 10 μM AVP. Mean values of triplicate determinations are presented. Standard error values of specific binding (△) are given. The experiment was repeated with similar results.

$d(CH_2)_5$Tyr(Me)AVP was 10-fold less potent than the selective V_2 antagonist $d(CH_2)_5$D-IleVAVP (data not shown). These data indicate that [³H]-SK&F 101926 bound with high affinity to V_2 vasopressin receptors.

Discussion

Vasopressin receptors have been studied with radiolabeled agonists. Here we report the receptor-binding properties of the radiolabeled vasopressin antagonist [³H-Phe]-desGlyd-$(CH_2)_5$D-Tyr(Et)VAVP, [³H]-SK&F 101926. desGlyd-$(CH_2)_5$D-Tyr(Et)VAVP was radiolabeled because this vasopressin analog is a potent antagonist at all vasopressin receptors studied (3, 3a, 4).

We studied the binding properties of [³H]-SK&F 101926 using the vascular smooth muscle cell line A-10, which expresses V_1 receptors (2, 11–14) and rat liver (15). V_2 receptors were studied using MDCK kidney cells. We found that [³H]-SK&F 101926 bound to plasma membranes of A-10 cells and to intact cells in monolayer in a specific, reversible, and saturable manner. At the radioligand concentrations used, [³H]-SK&F 101926 appeared to bind with a single high affinity. However, enhanced dissociation of [³H]-SK&F 101926 from intact cells in the presence of excess AVP or SK&F 101926 suggested negative cooperativity between the receptors. The affinity and the B_{max} of the binding sites determined on plasma membranes and intact cells were similar. Furthermore, the rank order potencies of vasopressin agonists and antagonists determined in competition binding experiments with [³H]-SK&F 101926 and [³H]AVP using plasma membranes of A-10 cells and liver membranes were the same. The rank order potency was typical for the V_1 vascular receptor subtype. [³H]-SK&F 101926 also bound specifically to the V_2 vasopressin receptors of MDCK cells. Therefore, [³H]-SK&F 101926 is a useful radioligand to study V_1 and V_2 receptors.

We investigated the effects of guanine nucleotides on the affinity of V_1 receptors in competition binding experiments using plasma membranes of A-10 cells and rat liver. Gpp(NH)p did not significantly alter the affinity of the V_1 antagonist $d(CH_2)_5$Tyr(Me)AVP, but decreased the affinity of AVP for the cell and liver receptors. These data support the hypothesis that the affinity of V_1 receptors can be regulated by guanine nucleotides (2, 16).

Acknowledgments

We would like to thank W. Bryan and K. Garnes and Drs. R. Heys, W. Kokke, and S. Senderoff for their assistance in the preparation of [³H]-SK&F 101926, and Mrs. C. Kelly for expert secretarial assistance.

References

1. Lefkowitz, R. J., A. L. DeLean, B. B. Hoffman, J. M. Stadel, R. Kent, T. Michel, and L. Limbird. Molecular pharmacology of adenylate cyclase-coupled alpha- and beta-adrenergic receptors. *Adv. Cyclic Nucleotide Res.* **14**:145–162 (1981).
2. Stassen, F. L., G. Heckman, D, Schmidt, N. Aiyar, P. Nambi, and S. T. Crooke. Identification and characterization of vascular (V_1) vasopressin receptors of an established smooth muscle cell line. *Mol. Pharmacol.* **31**:259–266 (1987).
3. Stassen, F. L., G. D. Heckman, D. B. Schmidt, J. Stefankiewicz, L. Sulat, W. F. Huffman, M. Moore, and L. B. Kinter. Actions of vasopressin antagonists: molecular mechanisms. In *Vasopressin* (R. W. Schrier, ed.). Raven Press, New York, 145–154 (1985).
3a. Ohlstein, E. H., and B. B. Berkowitz. Human vascular vasopressin receptors: analysis with selective vasopressin receptor antagonists. *J. Pharmacol. Exp. Ther.* **239**:737–741 (1986).
4. Kinter, L. B., J. Dubb, W. Huffman, F. Brennan, and F. L. Stassen. Potential role of vasopressin antagonists in the treatment of water-retaining disorders. In *Vasopressin* (R. W. Schrier, ed.). Raven Press, New York, 553–561 (1985).
5. Neville, D. M., Jr. Isolation of an organ specific protein antigen from cell-surface membrane of rat liver. *Biochim. Biophys. Acta* **154**:540 (1968).
6. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
7. Hancock, A. A., A. L. DeLean, and R. J. Lefkowitz. Quantitative resolution of beta-adrenergic receptor subtypes by selective ligand binding: application of a computerized model fitting technique. *Mol. Pharmacol.* **16**:1–9 (1979).
8. Kruszynski, M., B. Lammek, M. Manning, J. Seto, J. Haldar, and W. H. Sawyer. [1-β-Mercapto-β,β-cyclopentamethylene propionic acid), 2-*O*-methyl) tyrosine] arginine-vasopressin and [1-(β-mercapto-β,β-cyclopenta-

methylene propionic acid)] arginine-vasopressin, two highly potent antagonists of the vasopressor response to arginine-vasopressin. *J. Med. Chem.* **23**:364–368 (1980).
9. Manning, M., A. Olma, W. A. Klis, A. M. Kolodziejczyk, J. Seto, and W. H. Sawyer. Design of more potent antagonists of the antidiuretic responses to arginine vasopressin. *J. Med. Chem.* **25**:45–50 (1982).
10. Manning, M., W. A. Klis, A. Olma, J. Seto, and W. H. Sawyer. Design of more potent and selective antagonists of the antidiuretic responses to arginine vasopressin devoid of antidiuretic agonism. *J. Med. Chem.* **25**:414–419 (1982).
11. Nambi, P., N. Aiyar, M. Whitman, F. L. Stassen, and S. T. Crooke. Role of guanine nucleotide binding protein(s) in vasopressin-induced responses of a vascular smooth muscle cell line. *Fed. Proc.* **45**:1735 (1986).
12. Aiyar, N., P. Nambi, F. L. Stassen, and S. T. Crooke. Vascular vasopressin receptors mediate phosphatidylinositol turnover and calcium efflux in an established smooth muscle cell line. *Life Sci.* **39**:37–45 (1986).
13. Nambi, P., M. Whitman, G. Gessner, N. Aiyar, and S. T. Crooke. Vasopressin-mediated inhibition of atrial natriuretic factor-stimulated cGMP accumulation in an established smooth muscle cell line. *Proc. Natl. Acad. Sci. USA* **83**:8492–8495 (1986).
14. Nambi, P., M. Whitman, F. L. Stassen, and S. T. Crooke. Vascular vasopressin receptors mediate inhibition of beta adrenergic receptor-induced cyclic AMP accumulation. *J. Pharmacol. Exp. Ther.* **237**:143–146 (1986).
15. Keppens, S., and H. De Wulf. The nature of the hepatic receptors involved in vasopressin-induced glycogenolysis. *Biochim. Biophys. Acta* **588**:63–69 (1979).
16. Cantau, B., S. Keppens, H. De Wulf, and S. Jard. (^{3}H)-Vasopressin binding to isolated rat hepatocytes and liver membranes: regulation by GTP and relation to glycogen phosphorylase activation. *J. Recept. Res.* **1**:137–168 (1980).

Send reprint requests to: Dr. Frans L. Stassen, Department of Molecular Pharmacology, Smith Kline & French Laboratories, P. O. Box 7929, Philadelphia, PA 19101.