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Characterization of specific V_{1a} vasopressin-binding sites on a rat mammary-tumour-cell line

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WRK 1, a cloned cell line derived from a rat mammary tumour, carries specific vasopressin-binding sites. Specific binding of *2-tyrosine*-^{3}H-labelled [8-lysine]vasopressin ([^{3}H]vasopressin) was time-dependent, saturable and reversible. Scatchard-plot analysis of hormone binding indicated the presence of a single class of receptors with an equilibrium dissociation constant of 12.7 ± 0.2 nM. The maximal binding capacity was 75 ± 6 fmol/10^6 cells, which corresponds to approx. 45000 sites per cell. Oxytocin and a highly potent oxytocin analogue were able to inhibit completely [^{3}H]vasopressin binding, but, in this respect, they were far less potent than vasopressin. This clearly demonstrates the vasopressinergic nature of this receptor. Pharmacological studies using a series of 14 vasopressin or oxytocin analogues indicated that the ligand selectivity of the vasopressin receptor found on WRK 1 cells resembles that of the rat hepatocyte. This signifies that this vasopressin receptor is of the V_{1a} subtype. This conclusion was confirmed by the observation that vasopressin did not influence the production of intracellular cyclic AMP in WRK 1 cells.

INTRODUCTION

Recent studies with vasopressin clearly demonstrate that this hormone is more widely distributed in mammals than was previously assumed and that its actions are not limited to the well-established antidiuretic or pressor activities (for review, see ref. [1]). Thus, for example, vasopressin has been shown to elicit phosphorylase activation in liver [2], to modulate synaptic transmission in sympathetic ganglia [3], to act as a corticotropin-releasing factor in the neurohypophysis [4] and to stimulate mitosis in adrenal cells [5] and 3T3 cells [6]. Several vasopressin receptor subtypes can be distinguished. Michell *et al.* [7] have suggested a classification of vasopressin receptors based upon their transduction mechanisms [7]. Thus V_1 receptors are thought to mediate their intracellular effects by mobilizing Ca^{2+} in the cytosol (for a review, see ref. [2]), whereas V_2 receptors are coupled to the activation of adenylate cyclase [1]. Examination of the ligand selectivity of vasopressin receptors by using a large series of vasopressin structural analogues revealed marked differences between V_2 and V_1 receptors (for a review, see ref. [1]). Recent studies [8,9] have indicated that, on the basis of differences in their ligand selectivity, two subtypes of V_1 receptors can be distinguished and designated as 'V_{1a}' and 'V_{1b}' receptors. V_{1a} receptors are involved in the pressor, glycogenolytic and probably several other peripheral and central responses to vasopressin. V_{1b} receptors mediate vasopressin-induced corticotropin release by the adenohypophysis.

The coupling mechanism between the V_2 receptor and adenylate cyclase is now well established (for reviews, see refs. [1,10,11]). Until recently, much less was known about the coupling mechanism that links the V_1 receptor and the intracellular effectors that mediate the biological actions of vasopressin. However, evidence from a variety of tissues now suggests that occupancy of vasopressin V_1 receptors leads to a hydrolysis of the membrane phospholipid phosphatidylinositol 4,5-biphosphate (see [12–15] for reviews). The associated release of inositol 1,4,5-trisphosphate inside the cell serves as a second messenger to mobilize Ca^{2+} from endoplasmic-reticulum stores (see [16] for review), and accumulated diacylglycerol acts synergistically with Ca^{2+} to activate protein kinase C [17].

Up to now no convenient cellular system has been available to study the properties and functions of V_1 receptors. However, Monaco and co-workers [18,19] have described a cloned cell line (WRK 1), derived from a rat mammary tumour, in which vasopressin enhances protein accumulation and the incorporation of [^{14}C]-acetate into lipids. Vasopressin has also been shown to stimulate the metabolism of inositol lipids in these cells [20], and there is evidence for the existence of a discrete, hormone-sensitive, pool of an inositol lipid which is vulnerable to receptor-mediated degradation [21,22].

The aim of the present work was to characterize the vasopressin receptors present in WRK 1 cells and thus to define a cellular system in which to study the mechanism which couples vasopressin receptors to the as-yet-undefined molecules implicated in inositol-lipid hydrolysis.

MATERIALS AND METHODS

Chemicals

[^{3}H]Vasopressin was prepared as described previously [23]. It was purified by affinity chromatography on a

Abbreviations used: [^{3}H]vasopressin, *2-tyrosine*-^{3}H-labelled [8-lysine]vasopressin; MEM, Eagle's minimal essential medium; PBS, phosphate-buffered salt solution (full composition given in the text); vasopressin-analogue abbreviations are defined in Table 1; pK_d is $-\log[K_d(M)]$, K_d being the dissociation constant.

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Table 1. Vasopressin structural analogues used in the present study

References for the synthesis and biological activities of these peptides can be found in refs. [34–39]. Numbers in the left-hand column are those used in Fig. 4.

No.	Abbreviation	Full chemical name
1	AVP	[8-arginine]Vasopressin
2	desGly^{9}AVP	Des-9-glycine-[8-arginine]vasopressin
3	desGly^{9}d(CH_2)$_5$AVP	Des-9-glycine-[1-β-mercapto-β,β-cyclopentamethylenepropionic acid,8-arginine]-vasopressin
4	d(CH_2)$_5$[D-Phe^2]VAVP	[1-β-mercapto-β,β-cyclopentamethylenepropionic acid,2-D-phenylalanine,4-valine,8-arginine]Vasopressin
5	d(CH_2)$_5$[Tyr(Et)^2]VAVP	[1-β-mercapto-β,β-cyclopentamethylenepropionic acid,2-*O*-ethyltyrosine,4-valine,8-arginine]Vasopressin
6	desGly^{9}d(CH_2)$_5$[D-Tyr(Et)^2]-VAVP	Des-9-glycine-[1-β-mercapto-β,β-cyclopentamethylenepropionic acid,2-*O*-ethyl-D-tyrosine,4-valine,8-arginine]vasopressin
7	AVP acid	[8-arginine,9-glycine]Vasopressin
8	VDAVP	[4-valine,8-D-arginine]Vasopressin
9	dVDAVP	1-Deamino-[4-valine,8-D-arginine]vasopressin
10	OH-AVP	[1-L-2-hydroxy-3-mercaptopropanoic acid,8-arginine]Vasopressin
11	LVP	[8-lysine]Vasopressin
12	AVT	[8-arginine]Vasotocin
13	OT	Oxytocin
14	OH-Thr^4-Gly^7-OT	[1-(L-2-hydroxy-3-mercaptopropanoic acid),4-threonine,7-glycine]Oxytocin

neurophysin–Sepharose column. The radiochemical purity of the labelled peptide was checked by h.p.l.c. on a μBondapak C_{18} column (Waters Associates, Framingham, MA, U.S.A.). The peptide concentration of the purified hormone was determined by the Lowry method, with [8-lysine]vasopressin as a standard. The specific radioactivity of the labelled hormone was found to be 6.8 Ci/mmol. Its biological activity, determined by the rat kidney adenylate cyclase assay, was identical with that of the starting material (purified synthetic [lysine]-vasopressin). The vasopressin analogues used in the present study were generously given by Professor M. Manning, Medical College of Ohio, Toledo, OH, U.S.A., and are listed in Table 1.

Other chemicals were of the highest grade available and were obtained from the following sources: [^{3}H]adenine (24 Ci/mmol) from Amersham International; isobutylmethylxanthine, ATP, cyclic AMP and trichloroacetic acid from Sigma; forskolin from Calbiochem–Behring; [lysine]vasopressin from Bachem Switzerland.

Cell culture

WRK 1 cells were established and grown as previously described [19,20]. Cells were grown in monolayer cultures in MEM containing Earle's salts, glutamine (2 mM), penicillin (100 units/ml), streptomycin (100 mg/ml), fetal-calf serum (5%, v/v) and rat serum (2%, v/v). When confluent, cells were harvested with a solution of trypsin (0.05%) and EDTA (0.02%) and plated into Falcon plastic Petri dishes (35 mm-diameter) at a density of 7×10^4 cells in 1.5 ml of culture medium. Cultures were incubated at 37 °C in a humidified air/CO_2 (19:1) atmosphere. Cells adhered to the plastic substratum of the Petri dishes within 4–6 h and began to divide. The culture medium was replaced every 2–3 days. Cells were used after 5 days when the cell density was about $(5–7) \times 10^5$ cells/dish.

Determination of [^{3}H]vasopressin binding

Hormone binding was assayed on cells attached to the plastic substratum of the Petri dishes 5 days after seeding [24]. At the beginning of the experiment the culture medium was aspirated and cells washed with 3×2 ml of PBS which contained glucose (5.5 mM), $CaCl_2$ (0.44 mM), KCl (2.7 mM), KH_2PO_4 (1.5 mM), $MgCl_2$ (0.5 mM), NaCl (138 mM) and Na_2HPO_4 (8.1 mM), pH 7.4. Cells were then maintained at 37 °C. The hormone-binding reaction was initiated by rapidly aspirating the PBS solution and adding, to each Petri dish, 0.7 ml of PBS solution containing, in addition to glucose and the salts listed above, bovine serum albumin (1 mg/ml), tyrosine (1 mM) and various amounts of [^{3}H]vasopressin. Tyrosine was added to prevent any incorporation of [^{3}H]tyrosine originating from the possible hydrolysis of the *tyrosine*-^{3}H-labelled vasopressin. Cells were incubated at 37 °C in the presence of labelled hormone. The reaction was stopped by rapid removal of the incubation medium, followed by the addition of 2 ml of ice-cold PBS. Cells were rapidly detached by scraping with a rubber policeman. The cell suspension was layered on to the surface of a Gelman filter (Metricial membrane filter GA-3) under continuous aspiration. Petri dishes were rinsed with a further 2 ml of ice-cold PBS, which was transferred on to the filter. Filters were rinsed three times with 3 ml of ice-cold PBS. The whole procedure, including cell suspension and filtration, lasted 50 s. We have previously established that this procedure allows complete elimination of the free ligand without significant dissociation of the hormone–receptor complex [25,26]. The radioactivity retained on the filters was measured by liquid-scintillation spectroscopy. All determinations were performed in duplicate. In addition, for all the experimental series, non-specific binding was determined by incubating the cells in the presence of [^{3}H]vasopressin plus an excess of unlabelled vasopressin (10 μM). Specific binding was calculated as the difference between total and

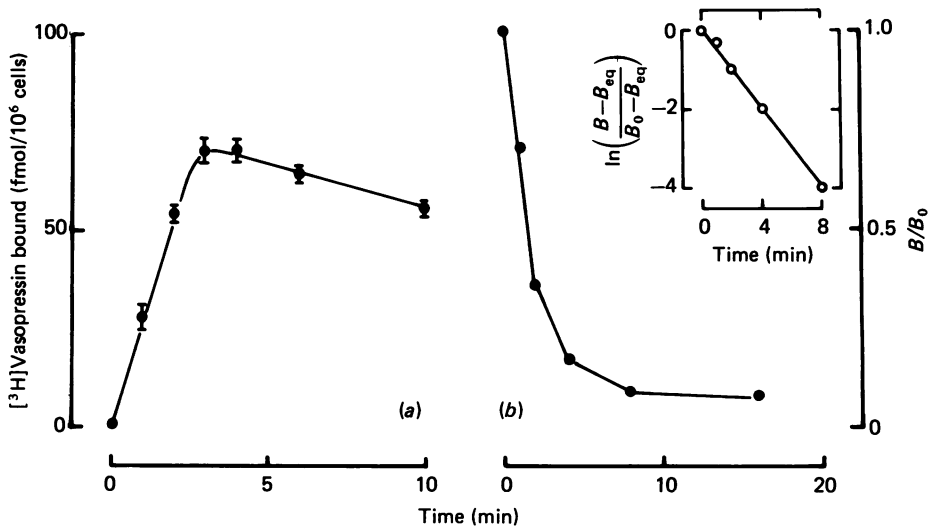

Fig. 1. Time course of [^{3}H]vasopressin binding to WRK 1 cells

(*a*) Association kinetics: WRK 1 cells were incubated at 37 °C in the presence of 10 nM-[^{3}H]vasopressin as described in the Materials and methods section and the radioactivity bound to the cells was measured by filtration. Results were corrected for non-specific binding (binding measured in the presence of 10 nM-[^{3}H]vasopressin plus 5 μM unlabelled vasopressin) and are means ± S.E.M. for three determinations. (*b*) Dissociation kinetics: WRK 1 cells were incubated for 6 min at 37 °C in the presence of 10 nM-[^{3}H]vasopressin. Unlabelled vasopressin (50 μM) was then added to the cells ($t = 0$) and the radioactivity which remained bound to the cells (B) was determined at the times indicated. Results are corrected for non-specific binding and are the means of two determinations. They are expressed as fractions of the specific binding measured at zero time (B_0). The semi-logarithmic plot of the dissociation curve $\ln(B-B_{eq})/(B_0-B_{eq})$ versus time is shown in the inset. The apparent equilibrium value for specific binding (B_{eq}) leading to the best exponential fit of the dissociation curve was determined empirically ($B_{eq}/B_0 = 0.079$).

non-specific binding and expressed as fmol of hormone bound/10^6 cells. The number of cells per Petri dish was determined by direct counting with a haemocytometer.

Control experiments have demonstrated that [^{3}H]vasopressin was not degradated by WRK 1 cells. Even after 12 min incubation at 37 °C in the presence of cells, the h.p.l.c. profile of labelled vasopressin was unmodified (results not shown).

The dissociation constant (K_d) of [^{3}H]vasopressin for its specific receptor was calculated as the concentration of labelled peptide leading to half-maximal specific binding. This value was derived by Scatchard analysis. The dissociation constants for unlabelled peptides were determined from competition experiments conducted as previously described [27]. Cells were incubated in the presence of a constant amount of [^{3}H]vasopressin (10 nM) and increasing amounts of the unlabelled analogue. The dissociation constant (K_d) of the unlabelled peptide was deduced by fitting the experimental data to the expected linear relationship:

$$\log[(B_0/B) - 1][(H^*/K_d^*) + 1] = \log[I] - \log K_d$$

where [I] is the concentration of unlabelled peptide, B is the specific vasopressin binding measured in the presence of unlabelled peptide, B_0 is the specific vasopressin binding measured in the absence of unlabelled peptide, K_d^* is the dissociation constant of [^{3}H]vasopressin, and H^* is the concentration of [^{3}H]vasopressin.

Assay of intracellular cyclic AMP

Intracellular cyclic AMP concentrations were determined by measuring the conversion of [^{3}H]adenine-nucleotide precursors into [^{3}H]cyclic AMP as previously described [28]. On day 5 in culture the cell medium was aspirated and replaced by fresh culture medium containing 2 μCi of [^{3}H]adenine (24 Ci/mmol/ml). After 1 h of incubation at 37 °C the cells were washed three times with 2 ml of PBS and incubated for 15 min at 37 °C in 1 ml of PBS containing glucose (5.5 mM) and isobutylmethylxanthine (1 mM). Hormones or other agents were then added to the medium. After 6 min incubation at 37 °C, the reaction was stopped by aspirating the medium and adding 1 ml of ice-cold 5% (w/v) trichloracetic acid. Cells were scraped off the plastic substratum with the aid of a rubber policeman, and unlabelled ATP and cyclic AMP were added to the mixture to a final concentration of 5 mM. The cellular extract was centrifuged at 5000 *g* for 15 min, and adenine nucleotides in the supernatant were separated by sequential chromatography on Dowex (Cl^- form) and alumina columns [29]. Cyclic AMP formation was expressed as the percentage conversion of [^{3}H]ATP into [^{3}H]cyclic AMP.

RESULTS

Binding of [^{3}H]vasopressin to WRK 1 cells

The results in Fig. 1 show that WRK 1 cells have specific binding sites for [^{3}H]vasopressin and that maximum binding was established within 3 min for a [^{3}H]vasopressin concentration of 10 nM. A small, but significant, decrease in specific binding was observed after longer periods of incubation. For this reason, an incubation time of 6 min with [^{3}H]vasopressin was adopted for subsequent experiments. Addition of an excess of unlabelled vasopressin (0.2 μM) to cells

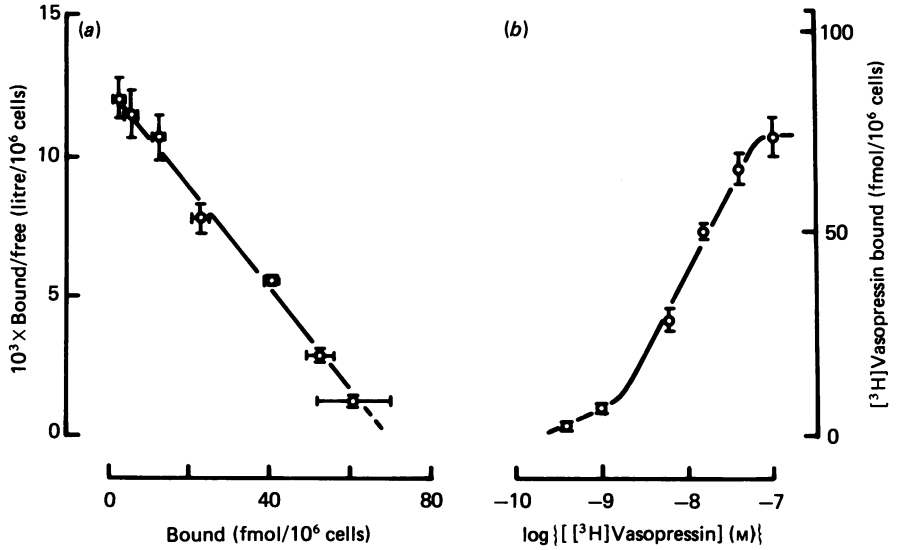

Fig. 2. Concentration-dependence of [^{3}H]vasopressin binding to WRK 1 cells

WRK 1 cells were incubated for 6 min at 37 °C in the presence of increasing amounts of [^{3}H]vasopressin. The radioactivity bound to the cells was measured as described in the Materials and methods section. Results were corrected for non-specific binding and are means ± S.E.M. for three separate experiments (*b*). (*a*) Shows a Scatchard plot of the results in (*b*). The values of the dissociation constant and maximal binding capacities estimated from the calculated regression lines are 12.3 nM and 79 fmol/10^6 cells respectively.

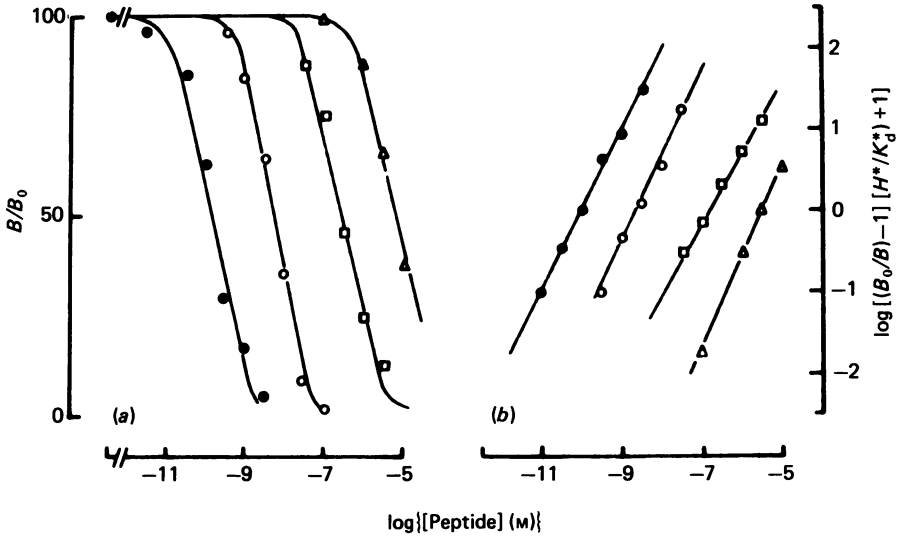

Fig. 3 Determination of dissociation constants for unlabelled peptides

WRK 1 cells were incubated for 6 min at 37 °C in the presence of 10 nM-[^{3}H]vasopressin and various concentrations of unlabelled peptide: ○, AVP; ●, desGly^{9}d(CH$_2$)$_5$[D-Tyr(Et)^2]VAVP; □, dVDAVP; △, AVP acid. Values for the specific binding of [^{3}H]vasopressin measured in the presence of unlabelled peptide (*B*) were expressed as a fraction of the specific binding measured in the absence of competitor (B_0). B/B_0 values are means from two separate experiments (*a*). The dissociation constants of unlabelled peptides (K_d) were deduced by fitting the experimental data to the expected linear relationship:

$$\log[(B_0/B)-1][(H^*/K_d^*)+1] = \log[I]-\log K_d$$

(see the Materials and methods section). pK_d values were calculated by regression analysis (*b*). The pseudo Hill coefficients of the displacement curves were: 1.01 (●), 0.74 (○), 1.16 (□) and 0.89 (△).

preincubated for 6 min in the presence of 10 nM-[^{3}H]vasopressin led to a rapid decrease in specific binding to the cells (Fig. 1*b*). The dissociation of bound [^{3}H]vasopressin followed an exponential time course. Half-maximal dissociation was achieved within 1.3 min (inset to Fig. 1*b*).

The [^{3}H]vasopressin binding to WRK 1 cells depicted in Fig. 2(*b*) was not fully saturated at the highest concentration of vasopressin tested (0.1 μM). However, non-specific binding at this point was very large (63% of total binding), thus precluding the use of higher concentrations of the hormone. Nonetheless, specific

Table 2. Binding to WRK 1 cells of peptides with enhanced selectivity for vasopressin or oxytocin receptors

Abbreviations used are indicated in the legend to Table 1. References for oxytocic and vasopressor activities are indicated in brackets. Values for pK_d are means ± S.E.M. of three or four separate determinations.

Peptide tested	pK_d for WRK 1 cells	Activity (units/mg)	
		Oxytocic	Vasopressor
AVP	8.42 ± 0.04	25.5	367 [37]
OH-AVP	9.00 ± 0.05	96	549 [34]
LVP	7.90 ± 0.01	22.1	270 [35]
AVT	8.54 ± 0.05	194	196 [36]
OT	6.14 ± 0.06	486	4 [37]
OH-Thr⁴-Gly⁷-OT	5.00 ± 0.08	1002	< 0.01 [38]

binding in the presence of 0.1 μM-vasopressin was not significantly greater than that observed with 0.04 μM-hormone, indicating that receptor occupation was approaching saturation. It was therefore appropriate to subject the data in Fig. 2(*b*) to further analysis by the method of Scatchard [30]. The Scatchard plot of these data is shown in Fig. 2(*a*) and indicates the presence of a homogeneous population of binding sites. The apparent dissociation constant, K_d, was 12.7 ± 0.2 nM (mean ± S.E.M. for three separate experiments). The maximal binding capacity, $B_{max.}$, was 75 ± 6 fmol/10⁶ cells (mean ± S.E.M. for three separate experiments). This value corresponds to 45000 sites per cell. The non-specific component of the binding represented 25 ± 3 and 63 ± 5% of the total binding for [³H]vasopressin concentrations of 6 and 100 nM respectively.

Pharmacological studies

In order to determine the pharmacological subtype of the vasopressin receptors present in WRK 1 cells we have measured the dissociation constant (K_d) of a series of vasopressin analogues. These peptides, which are listed in Table 1, were selected on the basis of their ability to discriminate between the different subtypes of vasopressin receptors already characterized. They were used in an unlabelled form and their respective affinities for WRK 1 cells were determined from competition experiments similar to those illustrated in Fig. 3. All the peptides tested inhibited [³H]vasopressin binding to the same maximal extent as did unlabelled vasopressin itself, indicating that they interacted with the entire population of binding sites labelled with [³H]vasopressin. The displacement curves were parallel, and complete displacement was obtained within a concentration range of about two orders of magnitude. [³H]Vasopressin was maximally displaced by 0.1 mM-[8-arginine]vasopressin and, in the presence of higher concentrations of this peptide, [³H]vasopressin binding remained constant. This presumably reflects non-specific binding. The other vasopressin analogues in Fig. 3 were not available in sufficient quantity to permit their use at concentrations higher than those indicated. The results in Fig. 3(*b*) indicate that the displacement curves could be fitted with a model involving reversible binding of both labelled and unlabelled peptide to a homogeneous population of binding sites. The relative affinities of the peptides tested for WRK 1-cell vasopressin receptors are given in Tables 2 and 3. WRK 1 cells exhibit a high affinity for the natural vasopressins in mammals and OH-AVP, a vasopressin analogue with enhanced pressor activity. Conversely, they bind oxytocin and the highly potent oxytocin analogue OH-Thr⁴-Gly⁷-OT with low affinity. These results establish the vasopressinergic character of the receptors. The results in Table 3 and Fig. 4 permit a comparison of the ligand selectivity of WRK 1 cell vasopressin receptors with that of the well-characterized V_{1a}, V_{1b} and V_2 receptors of rat liver, kidney and adenohypophysis respectively. This comparison demonstrates the close correlation between the pK_d ($-\log K_d$) values for a series of vasopressin analogues in WRK 1 cells and rat liver membranes. No such correlation was found with pK_d values determined on either rat kidney or rat adenohypophyseal membranes.

Adenylate cyclase activation in WRK 1 cells

The results in Table 4 illustrate the effects of various agents upon the conversion of intracellular ATP into cyclic AMP in WRK 1 cells. Forskolin and cholera toxin,

Table 3. Ligand selectivity of vasopressin receptors from WRK 1 cells: comparison with V_{1a}, V_{1b} and V_2 vasopressin receptors

Abbreviations used are indicated in Table 1. Affinity constants for WRK 1 cells are expressed in terms of pK_d values and are the means ± S.E.M. of three or four separate experiments similar to those illustrated in Fig. 3. The corresponding values for rat liver (V_{1a} receptor), rat kidney (V_2 receptor) and rat adenohypophyseal membranes (V_{1b} receptor) are taken from reference [9]. Abbreviation used: ND, not determined.

Peptide tested	pK_d			
	WRK 1 cells	Liver	Kidney	Hypophysis
AVP	8.4 ± 0.04	8.5	9.4	8.4
AVP acid	5.5 ± 0.1	5.7	7.2	4.7
desGly⁹AVP	6.4 ± 0.1	6.4	8.5	5.5
dVDAVP	6.9 ± 0.1	6.5	9.6	ND
VDAVP	6.2 ± 0.02	6.5	9.6	ND
desGly⁹d(CH_2)$_5$AVP	9.7 ± 0.05	9.6	7.0	5.7
d(CH_2)$_5$[D-Phe²]VAVP	8.8 ± 0.04	8.6	9.5	7.1
desGly⁹(CH_2)$_5$(D-Tyr(Et)²]VAVP	10.0 ± 0.01	9.7	9.9	5.7
d(CH_2)$_5$[Tyr(Et)²]VAVP	9.4 ± 0.1	9.5	9.5	5.8

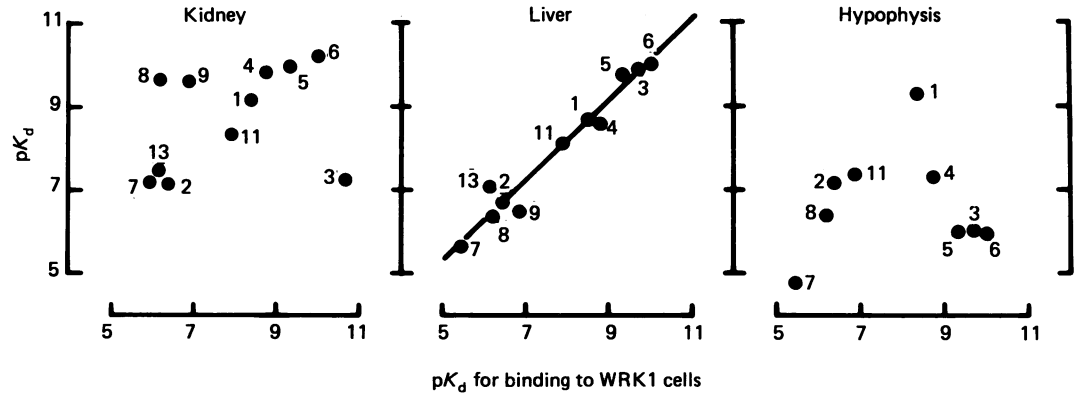

Fig. 4. Comparison of the ligand selectivity of WRK 1 cell vasopressin receptors with that of receptors from rat liver, rat kidney and rat adenohypophyseal membranes

The pK_d values for the binding to WRK 1 cells of [8-arginine]vasopressin and some of the vasopressin analogues listed in Table 1 are plotted as a function of the corresponding values determined on rat liver, rat kidney and rat adenohypophyseal membranes. The later values were taken from ref. [9]. A significant correlation was found only between data obtained on WRK 1 cells and liver membranes ($r = 0.977$, $P < 0.001$, $y = 1.01x - 0.3$). The key to the numbers used may be found in Table 1.

Table 4. Effect of vasopressin on cyclic AMP (cAMP) production by WRK 1 cells

Conversion of intracellular ATP into cyclic AMP was measured as described in the Materials and methods section. For experiments with cholera toxin, cells were treated for 24 h before the experiment with 10 μg of toxin/ml of culture medium. Values are means ± S.E.M. of six determinations derived from two separate experiments.

Addition	$\frac{[^3H]cAMP}{[^3H]cAMP+[^3H]ATP} \times 100$
None	0.142 ± 0.011
Vasopressin (100 μM)	0.171 ± 0.003
Forskolin (50 μM)	1.700 ± 0.070
Cholera toxin (10 μg/ml)	0.696 ± 0.010

which are known to activate adenylate cyclase in various systems, stimulated the production of cyclic AMP by factors of 12 and 4.9 respectively. However, vasopressin at a concentration (10 μM) that evokes almost complete occupation of its specific binding sites did not cause any significant activation of cyclic AMP production.

DISCUSSION

The present data clearly demonstrate the existence of specific vasopressin binding sites on WRK 1 cells. Binding of [^{3}H]vasopressin to WRK 1 cells is reversible, and the population of receptor sites is homogenous, as indicated by the linear Scatchard plot of the binding experiments in Fig. 2. The apparent affinity of this receptor for [^{3}H]vasopressin was similar to that of other vasopressin receptors originating from various tissues or species (for a review, see ref. [1]). The maximal vasopressin binding capacity of WRK 1 cells (45000 sites per cell) is of the same order of magnitude as the maximal vasopressin binding capacities found in rat aortic myocytes (24000) [24] and the pig kidney cell line LLC-PK$_1$ (60000) [11]. However, this density was lower than that detected on rat hepatocytes (200000) [27]. Vasopressin-binding sites could also be detected on partially purified membranes from WRK 1 cells (results not shown). A maximal binding capacity of 0.85 ± 0.14 pmol of [^{3}H]vasopressin bound/mg of protein was determined. This value is within the range of vasopressin-receptor densities measured on other membrane preparations (for review, see ref. [1]).

In principle, vasopressin receptors may be classified according to at least two criteria: (*a*) their ligand selectivity with respect to a series of vasopressin analogues [1] and (*b*) the molecular mechanisms by which receptor activation is coupled to the biological actions of vasopressin within the cell [7]. In the present study we have focused our attention on the first criterion. Although WRK 1 cells were derived from a tumour of the rat mammary gland, an oxytocin-sensitive tissue, the receptors detected on these cells are clearly vasopressinergic in nature (Table 2). However, our results do not exclude the presence of oxytocin receptors on WRK 1 cells. Indeed, oxytocin receptors usually have a low affinity for vasopressin and, if present on WRK 1 cells, they would not have been labelled to any detectable extent with [^{3}H]vasopressin in the present study.

Like other vasopressin receptors from rat tissues, the receptors of WRK 1 cells have a slightly higher affinity for [8-arginine]vasopressin, the natural antidiuretic hormone in this species, than for [8-lysine]vasopressin [1]. Four of the vasopressin analogues which were tested, AVP acid, VDAVP, dVDAVP and desGly^{9}AVP, exhibit enhanced selectivity for V_2 (renal) receptors as compared with V_{1a} (hepatic and vascular) and V_{1b} (adenohypophyseal) receptors [9]. All bind to WRK 1 cells with low affinity (pK_d values ranging from 5.49 to 6.44). One analogue, desGly^{9}d(CH$_2$)$_5$AVP, was selected on the basis of its selectivity for V_{1a} versus V_{1b} and V_2 receptors [9]. It had a high affinity for WRK 1 cell receptors. Finally, the series of peptides tested also included four of the

vasopressin antagonists which allowed the characterization of the new V_{1b} receptor in the rat adenohypophysis [9]. All exhibit high and similar affinities for WRK 1 cells and rat liver membranes as compared with their very low affinities for rat adenohypophyseal membranes. Altogether these results demonstrate the existence of striking similarities between the ligand selectivity of vasopressin receptors present on WRK 1 cells and that of the V_{1a} receptors involved in the vasopressor, glycogenolytic and probably several other peripheral and central responses to vasopressin. WRK 1 cells represent a convenient cellular model for further studies on V_{1a} receptors. Our conclusion, that WRK 1 cells carry V_1 receptors only, is supported by the observation that vasopressin did not evoke significant cyclic AMP accumulation in these cells (Table 2).

The specific vasopressin-binding sites detected on WRK 1 cells are probably the physiological receptors which mediate the stimulation of protein and lipid synthesis by vasopressin in these cells [19]. This conclusion is supported by the correlation between the relative affinities of AVP and oxytocin for the receptor and the relative abilities of these peptides to stimulate acetate incorporation into lipids. Thus the affinity of oxytocin for WRK 1 vasopressin receptors and the EC_{50} (concentration of the hormone required to provoke half-maximal effect) for stimulation of lipid synthesis by this hormone are both 200–400-fold lower than the corresponding values for AVP (Table 2 and ref. [19]).

The dissociation constant for [8-arginine]vasopressin at the vasopressin receptor of WRK 1 cells (3.8 nM) is quite different from the EC_{50} of vasopressin-induced acetate incorporation into lipids (0.1–0.2 nM). This difference may be partly due to the different experimental procedures adopted for the determination of these two parameters: we incubated cells for 6 min at 37 °C in our binding assays, but, in the acetate-incorporation studies, cells were incubated for 36 h under similar conditions [19]. However, it is important to note that such discrepancies between the concentration of hormones which half-saturate receptor binding and those required to evoke half-maximal physiological responses are commonplace for ligands which act through cell-surface receptors. For example, the concentration of vasopressin required to cause maximal activation of hepatic phosphorylase will evoke occupation of less than 1% of the vasopressin receptors present on isolated hepatocytes [12,13,27]. Hence the 'receptor reserve' for vasopressin in WRK 1 cells is similar to that existing in other cell types.

The existence of these 'spare receptors' focuses attention on the mechanism that couples V_1 vasopressin-receptor occupation to the evocation of physiological responses within the cell. A considerable body of evidence, derived from a variety of systems including WRK 1 cells, suggests that this mechanism involves the breakdown of membrane inositol phospholipids and the associated release of lipid-derived second-messenger molecules into the cell [12–16,20–22,31,32]. These tissues are discussed in greater detail in the following paper [33].

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