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Comparative pharmacology of bovine, human and rat vasopressin receptor isoforms

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Abstract

In this study, we characterized the bovine vasopressin V_{1a}, V_{1b}, V_2 receptor isoforms and compared their pharmacological properties to those of corresponding rat and human vasopressin receptor subtypes. Specific binding sites of high affinity for vasopressin were found in all bovine tissues tested (kidney, liver and pituitary). Using a large series of recent peptidic and non-peptidic selective vasopressin agonists or antagonists, we demonstrated the presence of vasopressin V_2, V_{1a} or V_{1b} receptors in the kidney, liver and pituitary bovine tissues, respectively. This extensive characterization of bovine vasopressin receptor isoforms validates the pharmacological vasopressin receptor classification earlier established for the rat and human species. As expected, the bovine vasopressin receptors look much more like human receptors than rat ones. Interestingly, among the three vasopressin receptor isoforms studied, the vasopressin V_{1b} receptor subtype is the best conserved for the three species studied.

Keywords: Vasopressin receptor; Tissue, bovine; Comparative pharmacology

1. Introduction

In mammals, vasopressin, a small polypeptidic neurohypophyseal hormone, exerts various biological effects. At the periphery, its major physiological role is played in regulating water and solute excretion by the kidney (Jard et al., 1984). This hormone is also involved in blood pressure control, smooth muscle contraction, platelet aggregation, corticotropin and aldosterone secretion, hepatic glycogenolysis and uterine motility (Gillies et al., 1982; Gallo-Payet and Guillon, 1998). In the brain, vasopressin may act as a neurotransmitter or a neuromodulator in various physiological responses, such as thermoregulation and cardiovascular homeostasis, as well as in modulations of learning and memory (Dreifuss et al., 1991; Barberis and Tribollet, 1996). More recently, vasopressin was also shown to alter sexual and cognitive behaviours in vole (Insel et al., 1994) and to regulate central functions involved in stress disorders such as anxiety and depression (Griebel et al., 2002; Wersinger et al., 2002). These distinct biological functions are mediated by three different vasopressin receptor subtypes: V_{1a}, V_{1b} and V_2. This classification is based upon their coupling to distinct second messenger cascades and their pharmacological profiles for a series of vasopressin compounds (Jard et al., 1984).

In mammals, the vasopressin V_{1a} receptors are found principally in the liver and in vascular myocytes but also in a great number of other organs such as adrenals, heart, genital tractus and in the brain (Morel et al., 1992; Thibonnier et al., 1991). The tissular localization of the

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vasopressin V_{1b} receptors is restricted to pituitary (Jard et al., 1986), adrenal medulla (Grazzini et al., 1996), pancreas (Lee et al., 1995) but also to several brain regions (Lolait et al., 1995). Both vasopressin V_{1a} and V_{1b} receptors are positively coupled to phospholipase C and act via intracellular calcium mobilization and protein kinase C activation. In contrast, vasopressin V_2 receptors are mainly expressed in the kidney and are positively coupled to adenylyl-cyclase. They play a predominant role in the antidiuretic effect of vasopressin (for review, see Jard, 1998). The characterization of these vasopressin receptors in various tissues and cell types from different species has been made possible via the synthesis of numerous peptidic and non-peptidic vasopressin molecules and the development of many radiolabelled vasopressin compounds with high affinity and good specificity (for review, see Barberis et al., 1999). Cloning of the different human and rat vasopressin receptor subtypes (Lolait et al., 1992; Morel et al., 1992; Sugimoto et al., 1994) confirmed that these peptidic receptors belong to the family of the G-protein-coupled receptors (GPCRs) and validated the pharmacological classification earlier proposed.

Up to now, the pharmacology of vasopressin receptors has only been extensively studied in the rat and human species. These comparative data reveal marked species differences (Tahara et al., 1999). For example: 1-deamino[8-D-arginine]vasopressin (dDAVP), which is known to be a selective vasopressin V_2 receptor agonist in the rat, displays a better affinity for human vasopressin V_{1b} receptors and, thus, a mixed vasopressin V_{1b}/V_2 receptor pharmacological profile in this species (Saito et al., 1997). Conversely, (1-[L-2-hydroxy-3-mercaptopropionic acid), 2-phenylalanine,4-homoglutamine,8-(L-(N^{γ}-L-2-aminobutyryl)-2,4-diaminobutyric acid)]vasotocin (F-180) (Aurell et al., 1990), which behaves as a selective human vasopressin V_{1a} receptor agonist with nanomolar affinity, exhibits a lower affinity for rat vasopressin V_{1a} receptors and no vasopressin V_{1a} receptor selectivity (Andrés et al., 2002). Finally, 1-deamino[Cyclohexylalanine]vasopressin (d[Cha^4]AVP), which is a selective vasopressin V_{1b} receptor agonist in human is a mixed vasopressin V_{1b}/V_2 receptor agonist in the rat (Derick et al., 2002). Whether or not these strong species differences are specific to this class of GPCR remains an interesting pharmacological question. Some investigations performed by Hechter et al. (1969) seem to validate this assumption since the vasopressin V_2 receptor expressed in bovine kidney looks like the rat vasopressin V_2 receptors, yet with some differences. Vasopressin receptors present in bovine adrenal medulla exhibit a vasopressin V_1 receptor pharmacological profile, but have been poorly studied (Nussey et al., 1987).

This study focusses on an extensive characterization of the different vasopressin receptors isoforms expressed in bovine tissues and a comparison of their pharmacological profiles with those of rat and human vasopressin receptors.

2. Materials and methods

2.1. Chemicals

Most of the standard chemicals were purchased from Sigma (St. Louis, MO, USA), Boehringer Mannheim (Mannheim, Germany), or Merck & Co. (Darmstadt, Germany), unless otherwise indicated. Tritiated Arg^8 vasopressin ([^{3}H]AVP, 80 Ci/mmol) were from Perkin Elmer Life Sciences (Courtaboeuf, France). [HO]Phenylacetyl-D-$Tyr(Me)^2$-Phe^3-Gln^4, Asn^5, Arg^6-Pro^7-Arg^8-NH^2 (HO-LVA), a specific vasopressin V_{1a} receptor antagonist was radioiodinated (2000 Ci/mmol) leading to [^{125}I]HO-LVA as reported by Barberis et al. (1999).

The formula, abbreviations and references for each vasopressin compounds used in this study are listed in Table 1. Vasopressin, oxytocin and most of the peptides used in this study were supplied by Bachem (Bubendorf, Switzerland). d$(CH_2)_5$[$Tyr(Me)^2$]AVP and d[Cha^4]AVP were sythesized by M. Manning and co-workers (Medical College of Ohio, Toledo, OH, USA). SR49059, SR121463 and SSR149415 were obtained from C. Serradeil-Le Gal (Sanofi Synthelabo Recherche laboratories, Toulouse, France). F-180, a structural compound of [Phe^2, Orn^8]VT, was from Ferring Research Institute (San Diego, CA, USA). All compounds were first dissolved in a millimolar stock solution in dimethyl-sulphoxide (DMSO) and diluted in the appropriate incubation medium. The final concentration of DMSO in the binding assay medium never exceeded 0.1% (v/v), a concentration which did not affect specific vasopressin binding (data not shown).

GF/C glass-fibre filters used were obtained from Whatman (Whatman International, Maidstone, UK).

2.2. Isolation of plasma membranes

Liver, kidney and pituitary glands were collected at a local slaughterhouse, placed in ice-cold Hank's balanced salt solution (HBSS) and transported immediately to the laboratory. Glands were cleaned of adhering fat and immediately processed. Animal manipulations were performed according to the recommendation of the French Ethical Committee.

Plasma membranes were isolated as previously described by Trueba et al. (1991) and used immediately or kept at −20 °C in the presence of 40% v/v glycerol until use.

Protein concentration was measured with the Bio-Rad protein assay kit and using bovine serum albumin as a standard.

2.3. Binding assays

Binding assays were performed as previously described (Grazzini et al., 1996). Briefly, plasma membranes (15–20 μg protein for liver and 40–60 μg protein for pituitary and kidney) were incubated in 200 μl of a medium containing:

Table 1
Structure and pharmacological properties of vasopressin-related compounds tested

Structure	Abbreviation	Pharmacological properties
• [8-Arginine]vasopressin (Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2)	VP[a]	Natural hormone
• Oxytocin (Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly- NH_2)	OT[a]	Natural hormone
• 1-Deamino[4-Valine,8-D-arginine]vasopressin	dVDAVP[b]	V_2 agonist
• 1-[4-(*N*-*tert*-butylcarbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro[4-(2-morpholinoethoxy)cyclohexane]indol-2-one, fumarate	SR121463[c]	V_2 antagonist
• 1-Deamino[8-D-arginine]vasopressin	dDAVP[d]	V_{1b}/V_2 agonist
• (1-[L-2-hydroxy-3-mercaptopropionic acid), 2-phenylalanine,4-homoglutamine, 8-(L-(N^{γ}-L-2-aminobutyryl)-2,4-diaminobutyric acid)]vasotocin	F-180[e]	V_{1a} agonist
• [1-(β-Mercapto-cyclopentamethylene propionic acid), 2-(*O*-methyl)tyrosine,8-arginine] vasopressin	$d(CH_2)_5$[Tyr(Me)^2]AVP[f]	V_{1a} antagonist
• ((2*S*) 1-[2*R*,3*S*)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]pyrrolidine-2-carboxamide	SR49059[c]	V_{1a} antagonist
• 1-Deamino[2-D-Phenylalanine,8-arginine]vasopressin	d[D-Phe^2]AVP[g]	V_{1a}/V_{1b} agonist
• 1-Deamino [2-D-3-(3′ -pyridyl)-alanine,8-arginine]vasopressin	d[D-3-Pal]VP[h]	V_{1a}/V_{1b} agonist
• 1-Deamino[Cyclohexylalanine]vasopressin	d[Cha^4]AVP[g]	V_{1b} agonist
• (2*S*,4*R*)-1-[5-Chloro-1-[(2,4-dimethoxyphenyl)sulfonyl]-3-(2-methoxy-phenyl)-2-oxo-2,3-dihydro-1*H*-indol-3-yl]-4-hydroxy-*N*,*N*-dimethyl-2-pyrrolidine carboxamide; isomer (–)	SSR149415[i]	V_{1b} antagonist

[a] Barberis et al. (1999).
[b] Manning et al. (1973).
[c] Serradeil-Le Gal et al. (2002b).
[d] Zaoral et al. (1967).
[e] Aurell et al. (1990).
[f] Kruszynski et al. (1980).
[g] Derick et al. (2002).
[h] Schwartz et al. (1991).
[i] Serradeil-Le Gal et al. (2002a).

50 mM Tris–HCl, pH 7.4, 2 mM $MgCl_2$, 1 mg/ml bovine serum albumin, 0.01 mg/ml leupeptine with increasing amounts of [^{125}I]HO-LVA (liver and pituitary) or [^{3}H]AVP (pituitary and kidney) for saturation experiments or with 30 pM [^{125}I]HO-LVA (liver) or 3–10 nM [^{3}H]AVP (pituitary and kidney), and varying concentrations of unlabelled peptides for competition studies. The reaction was allowed to proceed at 37 °C for 60 min for [^{125}I]HO-LVA and 45 min for [^{3}H]AVP. Non-specific binding was determined in each experimental condition by adding 10 μM of unlabelled vasopressin to the incubation medium.

Bound and free radioactivity were subsequently separated by vacuum filtration over Whatman GF/C filters, presoaked for at least 2 h either in 20 mg/ml bovine serum albumin ([^{3}H]AVP) or in 0.5% polyethylenimine ([^{125}I]HO-LVA) and radioactivity retained on the filters measured. Specific binding was calculated as the difference between total and corresponding non-specific binding. Affinities (K_d) for [^{125}I]HO-LVA, as well as for [^{3}H]AVP, were directly determined from saturation binding experiments. Affinities (K_i) for unlabelled ligands were determined from competition experiments using [^{125}I]HO-LVA and [^{3}H]AVP as radioligands.

The radioligand binding data were analysed by GraphPad PRISM (GraphPad Software, San Diego, CA, USA). K_d (dissociation constant) and B_{max} (maximal binding capacity) values of radiolabelled compounds were determined from the Scatchard transformations of the saturation binding experiments. The inhibitory dissociation constant of unlabelled compounds (K_i) was calculated using the Cheng and Prusoff equation (1973).

2.4. Autoradiography

Localization of vasopressin binding sites was performed according to a previously published protocol (Barberis et al., 1999). Briefly, pituitary glands were cleaned from adhering fat, quickly frozen by immersion in isopentane at −40 °C, and stored at −80 °C until used. Frozen tissues were mounted in chucks and serial sections (20 μm thick) cut with a cryostat microtome (Jung CM 3000), mounted on poly-lysine-coated glass slides and kept at −80 °C until use. Sections, brought to room temperature, were preincubated for 15 min in buffer containing: 50 mM Tris–HCl, pH 7.4, 1 mM EDTA, 1 mg/ml bovine serum albumin, in order to dissociate endogenous vasopressin which might have remained bound to vasopressin receptors, and then rinsed once for 2 min in a washing medium containing: 50 mM Tris–HCl, pH 7.4, 2 mM $MgCl_2$, 1mM EDTA and 1 mg/ml bovine serum albumin. Binding was carried out by covering each slide with 500 μl of the incubation medium containing: 50 mM Tris–HCl, pH 7.4, 2 mM $MgCl_2$, 1mM EDTA, 1 mg/ml bovine serum albumin and 0.01 mg/ml leupeptine and 10 nM [^{3}H]AVP either alone (total binding) or in the presence of the unlabelled compound tested. Non-specific binding was determined by incubating the adjacent section

in the same medium supplemented with 10 μM of unlabelled vasopressin. Incubation lasted 2 h at room temperature in a humid chamber under gentle agitation. It was followed by three 5-min washes in ice-cold medium (50 mM Tris–HCl, pH 7.4, 2 mM $MgCl_2$, 1 mg/ml bovine serum albumin) and a quick rinse in ice-cold distilled water. The slides labelled with [^{3}H]AVP were placed on a phosphor-imaging plate for 4 days. Autoradiography images were digitalized and quantified by computerized densitometry with a Bio-Image Analyser (BAS 2000, Fuji) with NIH Image Software (National Institutes of Health, USA) as described by Serradeil-Le Gal et al. (1996).

2.5. Data analysis

Statistical differences were assessed by paired and unpaired two-tailed Student's *t*-test using Prism's statistical program. Differences were considered significant when $P>0.05$.

Results were expressed as the mean±standard error of the mean (S.E.M.) of at least three distinct experiments performed in triplicate unless otherwise indicated.

3. Results

3.1. Pharmacological characterization of bovine vasopressin receptors

To characterize bovine vasopressin receptors, we decided to prepare crude plasma membranes from bovine kidney, liver and pituitary tissues supposed to naturally express, as for rat and human species, the vasopressin V_2, V_{1a} and V_{1b} receptor isoforms, respectively.

Binding experiments were carried out as previously described for vasopressin receptors expressed in rat tissue membrane preparations (see Materials and methods). A preliminary set of experiments was performed to determine the optimal binding conditions. Specific binding of [^{125}I]HO-LVA to bovine liver or pituitary plasma membranes and of [^{3}H]AVP to bovine kidney medulla and pituitary membrane preparations reached an equilibrium within 60 and 45 min at 37 °C, respectively (data not shown).

Whatever the plasma membrane preparations considered, specific binding obtained from equilibrium-saturation experiments using [^{3}H]AVP as radioligand were saturable (Fig. 1A,B). Scatchard analysis of the data gave linear plots consistent with the presence of a single class of high affinity and low capacity binding sites (Table 2). Similar results were obtained in bovine liver membranes using [^{125}I]HO-LVA (Fig. 1C; Table 2). In contrast, using this iodinated vasopressin compound on bovine anterior pituitary membranes, a curvilinear Scatchard plot was obtained suggesting the presence of at least two distinct binding sites (Fig. 1D). The first one corresponded to specific binding sites of high affinity and low capacity for [^{125}I]HO-LVA. The second category exhibited a much lower affinity but an enhanced maximal binding capacity (Table 2).

To determine the nature of the bovine vasopressin receptor isoform present in the different plasma membrane preparations tested, binding competition experiments were performed using a series of specific vasopressin compounds known to discriminate between each vasopressin receptor

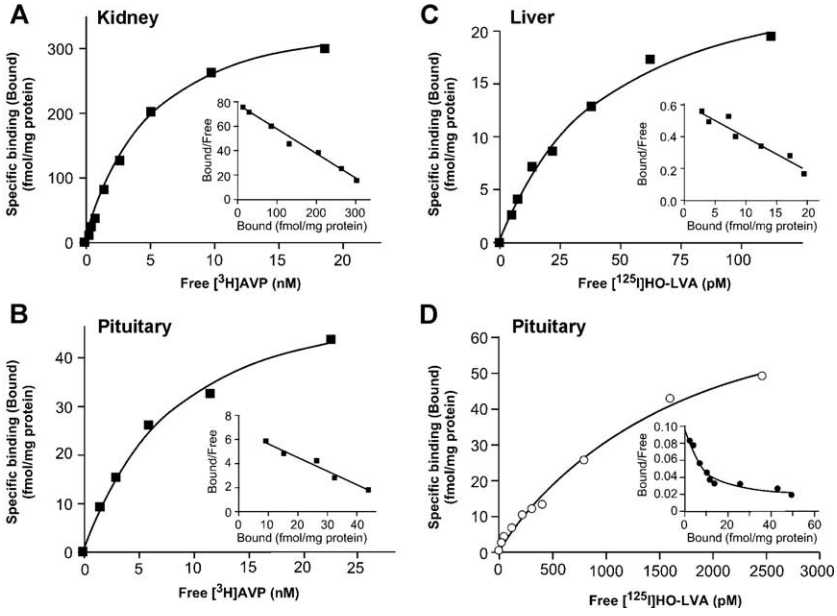

Fig. 1. Characterization of specific vasopressin binding sites from bovine tissues. Saturation binding experiments using [^{125}I]HO-LVA (liver and pituitary plasma membranes) or [^{3}H]AVP (pituitary and kidney plasma membranes) were performed as described in Materials and methods. Specific binding was calculated in each condition and plotted against the dose of radioligand used in the assay. Inset: Scatchard transformation of the data. Results illustrated are from one representative experiment of three to five, each performed in triplicate.

Table 2
Binding properties of vasopressin receptors from bovine tissues

Radioligand	Binding parameters	Kidney	Liver	Pituitary
[^{3}H]AVP	K_d (nM)	5.0±1.2	ND	10.3±0.5
	B_{max} (fmol/mg protein)	356±53	ND	88.6±15.9
[^{125}I]HO-LVA	K_{d1} (pM)	ND	33.0±9.2	96.2±4.1
	K_{d2} (pM)		–	883±177
	B_{max1} (fmol/mg protein)	ND	27.8±0.3	12.1±2.7
	B_{max2} (fmol/mg protein)		–	78.3±8.5

Binding experiments were performed as described in Materials and methods and are illustrated in Fig. 1. The affinity (K_d) and the maximal binding capacity (B_{max}) were deduced from saturation experiments using either [^{3}H]AVP or [^{125}I]HO-LVA as radioligand (see Fig. 1). Results are the mean±S.E.M. of three to five independent experiments performed on distinct membrane preparations.
ND, not determined.

subtypes and earlier characterized in several rat and human tissues.

For these studies, we used classical peptidic vasopressin compounds such as d(CH$_2$)$_5$[Tyr(Me)^2]AVP (Manning compound) and dDAVP (desmopressin) and non-peptidic compounds such as SR49059 and SR121463 known for their vasopressin receptor isoform selectivity (see Table 1). We also tested the two specific vasopressin V_{1b} receptor compounds recently described, d[Cha^4]AVP and SSR149415, and the specific vasopressin V_{1a} receptor agonist F-180. As illustrated in Fig. 2, all the unlabelled vasopressin compounds tested dose-dependently inhibited specific [^{125}I]HO-LVA (liver and pituitary vasopressin receptor) or [^{3}H]AVP (pituitary and kidney vasopressin receptor) binding up to non-specific values. Inhibition constants (K_i) were calculated as described in Materials and methods and summarized in Table 3.

As previously observed for the rat and human vasopressin receptors, [^{3}H]AVP exhibited a high affinity for the kidney, liver and pituitary membranes. Moreover, the K_i values deduced for unlabelled vasopressin from competition experiments fit very well with those determined from saturation binding experiments using [^{3}H]AVP as a radioligand (Tables 2 and 3) validating our binding assay conditions.

Results depicted in Table 3 show clear differences in terms of affinities for the different vasopressin compounds tested depending on the plasma membrane preparations considered.

The vasopressin V_2 receptor subtype present on bovine kidney membranes exhibited a high affinity for dDAVP and SR121463 (normalized selectivity index similar to that of

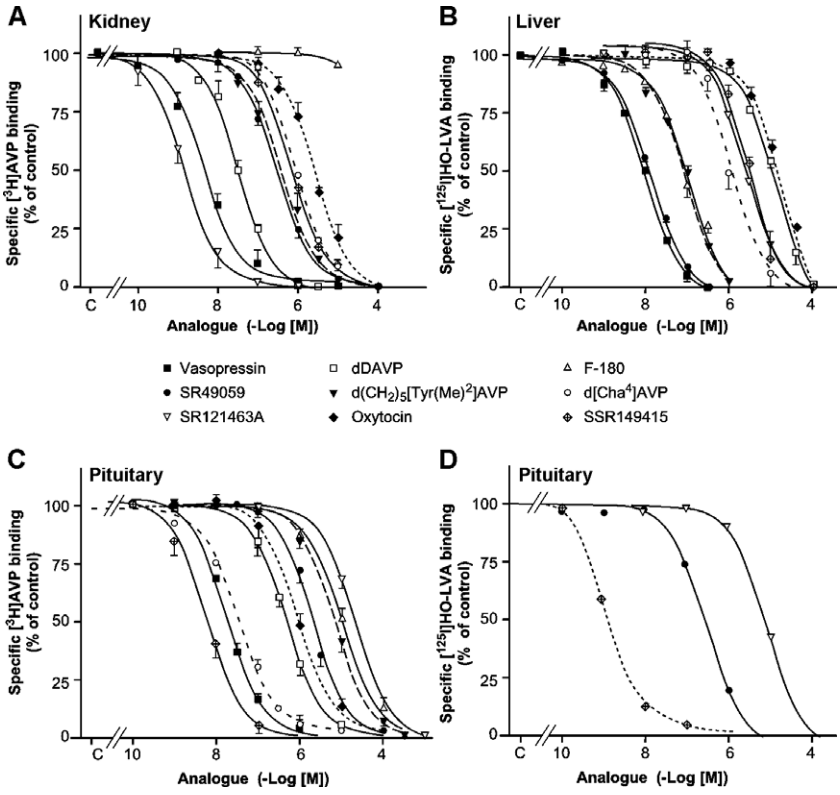

Fig. 2. Pharmacology of vasopressin binding sites from bovine tissues. Binding competition assays were performed in the presence of 30 or 300 pM [^{125}I]HO-LVA (liver or pituitary plasma membranes, respectively) or 3–5 nM [^{3}H]AVP (pituitary and kidney plasma membrane, respectively), as described in Materials and methods with or without (control, C) increasing amounts of unlabelled vasopressin compounds. Specific binding was calculated in each condition. Results are expressed as percent of control specific binding. Each point represents the mean±S.E.M. of four to eight independent experiments each performed in triplicate.

Table 3
Affinity of vasopressin compounds for bovine vasopressin V_{1a}, V_{1b} and V_2 receptors

Vasopressin compounds	Bovine liver vasopressin receptor[a]		Bovine pituitary vasopressin receptor[b]		Bovine kidney vasopressin receptor[b]	
	K_i (nM)	S.I.	K_i (nM)	S.I.	K_i (nM)	S.I.
Vasopressin	10.4±2.7	1.0	12.4±0.6	1.0	4.0±0.9	1.0
Oxytocin	(60±3%)	–	600±10	48	2270±450	567
dVDAVP	520±120	50	(73±2%)	–	1.6±0.4	0.4
SR121463	2017±440	194	(68±4%)	–	1.3±0.5	0.3
dDAVP	(54±2%)	–	282±158	23	22.5±1.0	5.6
F-180	56.2±12.8	5.4	6377±1872	514	(95±1%)	–
[d$(CH_2)_5$ Tyr(Me)^2]AVP	60.8±8.6	5.8	5167±420	417	397±169	99
SR49059	11.9±1.3	1.1	1055±55	85	386±132	96
d[D-Phe^2]AVP	13.4±2.3	1.3	5.8±1.8	0.5	849±68	212
d[D-3-Pal]VP	1750±420	168	515±115	41	9044±4150	2261
d[Cha^4]AVP	1333±391	128	24.5±4.9	2.0	712±35	178
SSR149415	3500±1000	336	2.7±0.8	0.2	592±95	148

Binding assays were performed as described in Materials and methods on bovine liver, pituitary and kidney plasma membrane preparations. Inhibition constants (K_i in nM) were determined from competition experiments.
Values are the mean±S.E.M. of four to six independent determinations each performed in triplicate. For compounds exhibiting affinities higher than 10 μM, the percentage of specific binding observed at this concentration was indicated in parentheses.
S.I. (normalized selectivity index)=K_i compound for V_x receptors/K_i AVP for V_x receptors.
[a] Competition experiments with [^{125}I]HO-LVA.
[b] Competition experiments with [^{3}H]AVP.

vasopressin). Vasopressin binding sites present in bovine liver membrane preparations showed a good affinity for the classical selective non-peptidic vasopressin V_{1a} receptor antagonist, SR49059 (normalized selectivity index close to that of vasopressin), and also for F-180 and d$(CH_2)_5$[Tyr(Me)^2]AVP, two selective peptidic human vasopressin V_{1a} receptor agonists. Bovine liver vasopressin receptors also exhibited an excellent affinity for d[D-Phe^2]AVP, previously shown to exhibit a mixed vasopressin V_{1a}/V_{1b} receptor profile for rat and human receptors (normalized selectivity index=1.3).

As bovine pituitary membranes exhibited at least two different categories of specific binding sites, their pharmacological characterization was more complex. The binding sites characterized by a low affinity for [^{125}I]HO-LVA and high capacity (site 2) corresponded to the vasopressin V_{1b} receptor subtype since (i) its affinity for SSR149415, measured in the presence of 300 pM of [^{125}I]HO-LVA was excellent (K_i=1.3±0.1 nM, four determinations) (Fig. 2, panel D) and similar to that found at human vasopressin V_{1b} receptors stably expressed in CHO cells (Derick, unpublished results), and (ii) SR121463 and SR49059, two specific vasopressin V_2 and V_{1a} receptor non-peptidic antagonists, respectively, exhibited a low affinity for these binding sites (K_i=4500±300 and 425±120 nM, respectively, three distinct determinations) (Fig. 2, panel D). The second binding site (site 1) exhibiting a higher affinity for [^{125}I]HO-LVA and a low capacity more probably corresponds to the bovine vasopressin V_{1a} receptor isoform since its K_i value for this iodinated vasopressin compound corresponds to that found for the bovine liver receptor (see Table 2). Competition binding experiments were also performed on bovine pituitary membranes using [^{3}H]AVP as a radioligand. The pharmacology of these binding sites correspond to that of vasopressin V_{1b} receptors since (i) their affinities for d[Cha^4]AVP and SSR149415, two specific vasopressin V_{1b} receptor compounds in mammals, were excellent (normalized K_i=2.0 and 0.2, respectively), and (ii) their affinities for specific vasopressin V_2 or V_{1a} receptor selective antagonists such as SR121463 and SR49059 were respectively higher than 1 μM (Table 3). Such results were also confirmed by results obtained with d[D-Phe^2]AVP, a mixed vasopressin V_{1a}/V_{1b} receptor compound. This compound exhibited a high affinity for bovine vasopressin receptors from liver and pituitary membranes (normalized selectivity index 1.3 and 0.5, respectively).

In contrast, as already found for rat and human vasopressin receptors, d[D-3-Pal]VP, supposed to be specific for vasopressin V_{1b} receptor isoforms (Schwartz et al., 1991), exhibited a weak vasopressin V_{1b} receptor selectivity and a low affinity for bovine pituitary vasopressin receptors (Table 3).

3.2. Autoradiographic localization of bovine vasopressin receptors

As plasma membranes from bovine pituitary express more than one type of vasopressin receptors (see above) and as some recent studies suggest the presence of vasopressin V_{1a} and V_{1b} receptors in rat pituitary (Orcel et al., 2002; Jard et al., 1986), we decided to study the tissular localization of vasopressin receptors in bovine pituitary. For this study, [^{3}H]AVP was used as a radioligand since it allows the labelling of all bovine vasopressin receptor isoforms (Table 3).

As illustrated in Fig. 3, an intensive [^{3}H]AVP heterogeneous labelling was observed on adenohypophysis. This

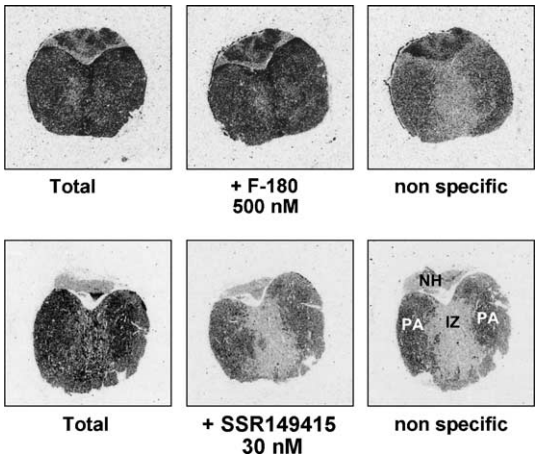

Fig. 3. Localization of vasopressin specific binding sites in bovine pituitaries. Autoradiograms were obtained from adjacent slices of bovine pituitary incubated with 10 nM [^{3}H]AVP alone (total binding) or in combination with 10 μM unlabelled vasopressin (non-specific binding) or with the appropriate unlabelled vasopressin compound tested (see Materials and methods). Slices were exposed 4–7 days in a phospho-imager and images digitalized. Illustrations are from one experiment representative of three. NH, neurohypophysis; PA and IZ, peripheric area and inner zone of the adenohypophysis, respectively.

total labelling was partially reversed by preincubating the pituitary slices with 10 μM unlabelled vasopressin suggesting the presence of specific vasopressin binding sites in some particular areas. Specific labelling was 3.3±0.4-fold more intense in the inner zone of the adenohypophysis than in the peripheric area, and represented 58±11% and 25±7% of total binding, respectively (n=4). To further characterize the vasopressin binding sites present in this organ, we preincubated bovine pituitary slices with different selective vasopressin compounds. Using 500 nM of unlabelled F-180, a specific vasopressin V_{1a} receptor antagonist (Table 3), we observed a small reduction of specific [^{3}H]AVP labelling (26±10%, n=3) both in the peripheric area and in the inner zone of the adenohypophisis (data not shown). Using 30 nM of unlabelled SSR149415, a specific vasopressin V_{1b} receptor antagonist, an almost complete inhibition of specific [^{3}H]AVP labelling was observed both in the inner zone and in the peripheric area (82±9%, n=3). Similar results were obtained using 200 nM of unlabelled d[Cha^4]AVP, a specific vasopressin V_{1b} receptor agonist (data not shown).

Quantification of [^{3}H]AVP labelling in the neurohypophysis indicated a small but significant specific binding, yet it represented only 13±8% of that found in the inner zone of adenohypophysis. For this reason, the pharmacological characterization of these binding sites was not possible.

3.3. Pharmacological profiles of bovine vasopressin receptors

As important species differences for vasopressin receptors have been earlier described, we compared the ligand-binding characteristics of bovine vasopressin receptors to those of rat and human.

The K_i values determined for a series of 12 unlabelled vasopressin compounds for bovine vasopressin receptor isoforms are listed in Table 3. Except as noted, corresponding values for human and rat vasopressin receptors were obtained with the same experimental protocol using membrane preparations from CHO cells stably transfected with the human vasopressin receptor or from native rat tissues known to express the vasopressin receptor isoform studied. Then, we plotted the pK_i values of vasopressin compounds determined for one bovine vasopressin receptor isoform against similar values derived from the rat or human corresponding vasopressin receptor subtype.

As shown in Fig. 4, panel A, an excellent correlation was observed when comparing the pharmacological profiles of bovine and human vasopressin V_2 receptors (r^2=0.9293, P<0.0001). A very high homology was also observed between the human and bovine vasopressin V_{1a} and V_{1b} receptors (r^2=0.7827, P=0.0007 and r^2=0.8469, P=0.0002, Fig. 4, panels D and G, respectively). These homologies were less pronounced when human and rat vasopressin receptors were compared (Fig. 4, panels C, F and I). The pharmacological profiles of rat and bovine vasopressin V_{1b} receptors were also very similar (Fig. 4, panel H). In contrast, rat and bovine vasopressin V_2 receptors showed weak pharmacological similarities (Fig. 4, panel B), as well as rat and bovine vasopressin V_{1a} receptors (Fig. 4, panel E).

4. Discussion

Using the recently discovered specific agonists and antagonists of each vasopressin receptor isoform, we pharmacologically characterized for the first time the vasopressin receptors expressed in native bovine tissues. We also compared their pharmacological profiles to those of corresponding rat and human vasopressin receptor isoforms.

In bovine, like in rat and human species, kidney specifically express vasopressin receptors of high affinity for [^{3}H]AVP and high density (Bichet, 1994). As previously described by Hechter et al. (1969) and further illustrated in this study, these bovine vasopressin receptors exhibit a typical vasopressin V_2 receptor pharmacological profile since they present a high affinity for specific vasopressin V_2 receptor agonists and antagonists like dDAVP and SR121463, respectively, and resemble human vasopressin V_2 receptors (Fig. 4, panel A).

Similarly, specific vasopressin receptor binding sites are also expressed in bovine liver tissues. As found for the rat and human, this receptor exhibits a nanomolar affinity for [^{3}H]AVP. Its density is relatively weak (Table 2) as compared to values found for rat liver vasopressin receptors but similar to those described for human liver tissues (Howl et al., 1991). The pharmacological profile of these bovine liver vasopressin receptors is typical of the vasopressin V_{1a}

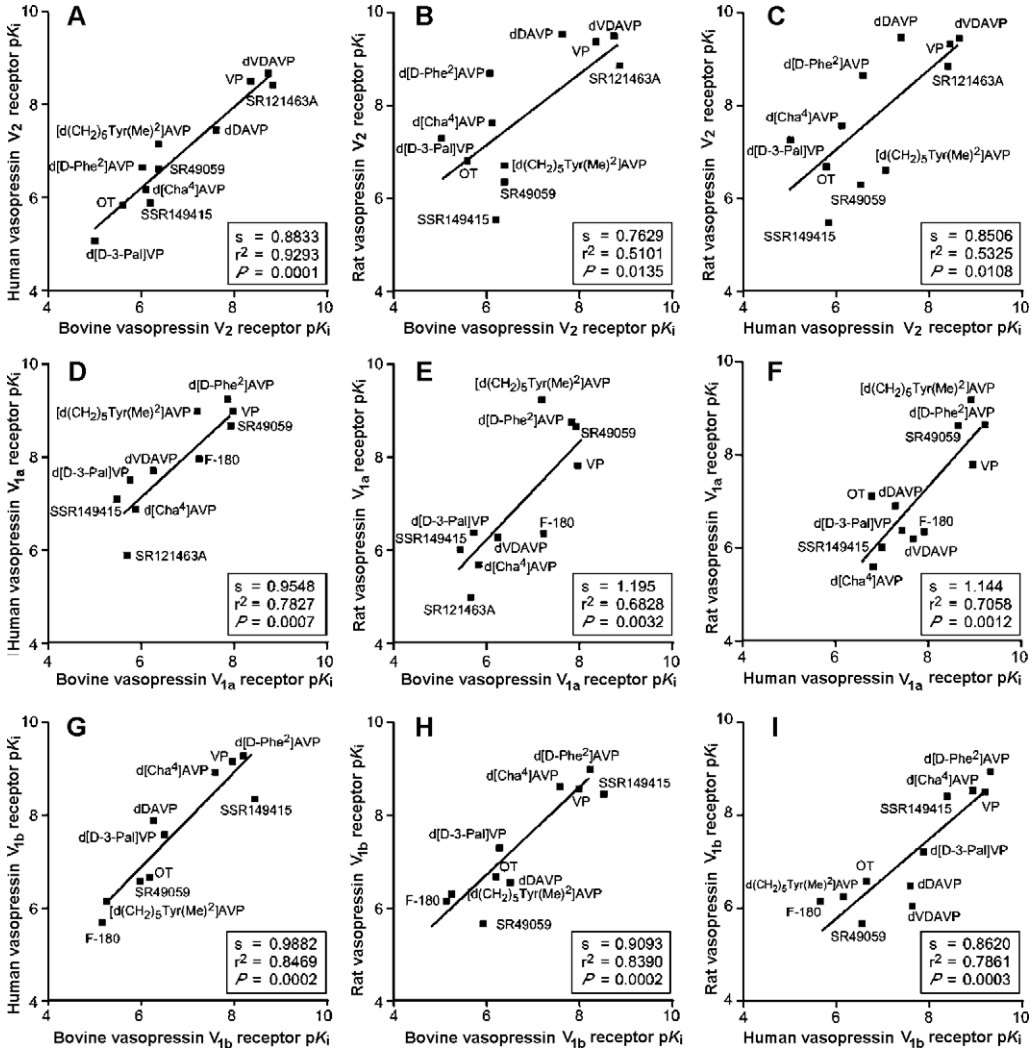

Fig. 4. Bovine, human and rat vasopressin receptor pharmacological profiles. For each vasopressin receptor isoform, (V_{1a}, V_{1b}, V_2), we plotted the pK_i (−Log K_i) of a series of vasopressin compounds for bovine vasopressin receptor against their corresponding pK_i values determined on rat and human. For rat and human vasopressin receptors, pK_i values were obtained using the same experimental protocol as that described in this study and previously published (Derick et al., 2002). Regression analysis of these data was performed and the slope of the regression curve (s), the correctness of fit (r^2) and p values (P) indicated in each case. See Table 1 for abbreviations.

receptor isoform. They present a good affinity for SR49059, F-180 and d$(CH_2)_5$[Tyr(Me)^2]AVP, three specific vasopressin V_{1a} receptor compounds (Table 3). Moreover, as illustrated in Fig. 4, panels D and E, the pharmacological pattern of bovine liver vasopressin receptors is like those of rat and human vasopressin V_{1a} receptors. Despite this good correlation, the affinities of bovine vasopressin V_{1a} receptors for these vasopressin compounds are 5- to 10-fold lower than those determined on human vasopressin V_{1a} receptor (left shift of the regression line as compared to the bisecting line, see Table 3 and Fig. 4, panel D).

Similar shift is also observed when we compared pK_i from rat and human vasopressin V_{1a} receptors (Fig. 4, panel F). Such discrepancies probably arise from the nature of the biological material used. For experiments dealing with rat and bovine receptors, we used plasma membranes from liver, a tissue known to express high amounts of proteolytic enzymes able to alter the binding properties of vasopressin receptors (Cantau et al., 1980) and from CHO cells, for experiments on human vasopressin V_{1a} receptors.

Bovine pituitary glands also express specific vasopressin binding sites. As found for the rat, these receptors exhibit a relatively good affinity for [^{3}H]AVP and a low binding capacity (Jard et al., 1986). Saturation binding experiments suggest the presence of more than one vasopressin receptor isoform in bovine pituitary membrane preparations. By using different vasopressin radioligands, one being able to bind to all vasopressin receptor isoforms ([^{3}H]AVP) and the other one able to discriminate between the vasopressin V_{1a} and V_{1b} receptor subtype ([^{125}I]HO-LVA), we found that the vasopressin V_{1b} receptor is mainly expressed in this tissue and represented 87% of total vasopressin specific binding sites present in bovine pituitary membranes preparation (see Table 2). Such an

assumption is confirmed by the fact that (i) displacement of [^{3}H]AVP specific binding by unlabelled vasopressin V_{1a} or V_{1b} receptor specific compounds like SR49059, F-180 or SSR149415 is Michaelian and not biphasic (Fig. 2), and (ii) d[Cha^4]AVP and SSR149415 (two selective vasopressin V_{1b} receptor compounds) but not F-180 or d$(CH_2)_5$[Tyr(Me)^2]AVP (two selective vasopressin V_{1a} receptor compounds) inhibit [^{3}H]AVP specific binding with a good affinity (Fig. 2; Table 3). The nature of bovine pituitary vasopressin V_{1b} receptors is further evidenced by comparing their pharmacological profile to that of rat and human vasopressin V_{1b} receptors (Fig. 4, panels G, H, I). Autoradiographic experiments also confirmed the predominance of vasopressin V_{1b} receptor isoform in bovine pituitary since (i) specific vasopressin V_{1b} receptor compounds like SSR149415 and d[Cha^4]AVP drastically reduced the specific [^{3}H]AVP labelling of the inner part of the adenohypophysis (Fig. 3), and (ii) specific vasopressin V_{1a} receptor agonists like F-180 (see Table 3) only weakly affected this labelling. Our autoradiographic experiments also allow to localize this vasopressin receptor within the pituitary. The vasopressin V_{1b} receptors are only present in the adenohypophysis and more particularly in its inner zone. Preliminary experiments (S. Derick, unpublished results) indicate that this area corresponds to the zone where corticotropin-releasing factor receptors are also expressed suggesting, as expected, the presence of vasopressin V_{1b} receptors on corticotrophs cells (Mason et al., 2002). The pharmacological characterization of vasopressin receptors exhibiting a high affinity for [^{125}I]HO-LVA was difficult due to their low and very variable maximal binding capacities from one preparation to another and to the weak K_d difference between the sites of low and high affinity (see Table 3). They probably corresponded to the vasopressin V_{1a} receptor isoforms since SR49059 tested in some preparations displaced [^{125}I]HO-LVA specific binding with a K_i of 7.5±1.7 nM (data not shown). These vasopressin receptors could correspond to vascular vasopressin V_{1a} receptors present on pituitary blood vessels since such isoforms have been previously characterized in rat myocytes (Vittet et al., 1986). However, we cannot exclude their presence on other secretagogue cells. Thus, Orcel et al. (2002) recently described on rat pituitary the presence of vasopressin V_{1a} receptors on gonadotroph cells using an antivasopressin V_{1a} receptor antibody. The existence of functional vasopressin V_{1a} receptors within the pituitary remains to be further confirmed since, according to these authors, these receptors are mainly present on intracellular cytoplasmic vesicules and not revealed by binding experiments performed on rat pituitary plasma membrane preparations using [^{3}H]AVP as a radioligand (Jard et al., 1986).

This extensive characterization of bovine vasopressin receptors reinforces the classification of this neuropeptide receptor family previously established for the rat and human species (for review, see Jard, 1998). The comparison of the pK_i of large series of vasopressin compounds for the different vasopressin receptor isoforms from different species is also very interesting. As illustrated in Fig. 4, the vasopressin V_{1b} receptor isoform is pharmacologically the best conserved among the three species studied. An excellent correlation coefficient is obtained between the pK_i of vasopressin compounds for rat versus bovine, rat versus human and human versus bovine (Fig. 4, panels G, H, I). In contrast, a more important variability of the vasopressin V_2 and the V_{1a} receptor pharmacological properties is observed among the three mammalian species studied (comparing panels A, B and C, and D, E, and F, respectively). Such observations may suggest that the physiological functions triggered by the vasopressin V_{1b} receptor, namely the regulation of the hypothalamus–pituitary–adrenal axis, are important. Recent studies showing a role of the vasopressin V_{1b} receptor in the control of stress and depression in rats may also reinforce this idea (Griebel et al., 2002).

This study also shows that the pharmacological profiles of bovine vasopressin receptors resemble more closely those of the corresponding human vasopressin isoforms than those of rat (comparing Fig. 4, panels A, D and G to C, F and I). Thus, along with the human transfected cell line models generally used for testing the pharmacological properties of new vasopressin compounds of potential interest for human applications, bovine native tissues may provide an alternative more physiological model.

In conclusion, this study validates the pharmacological classification of vasopressin receptors into three classes for bovine tissues and reveals that the vasopressin V_{1b} receptor subtype is the most conserved among the three mammalian species studied.

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