RESEARCH PAPER

Pharmacological characterization of FE 201874, the first selective high affinity rat V_{1A} vasopressin receptor agonist

Rafik Marir[1,2,3,4], Anne Virsolvy[5], Kazimierz Wisniewski[6], Julie Mion[1,2,3], Dominique Haddou[1,2,3], Evelyne Galibert[1,2,3], Zahia Meraihi[4], Michel G Desarménien[1,2,3] and Gilles Guillon[1,2,3]

[1]CNRS, UMR-5203, Institut de Génomique Fonctionnelle, Montpellier, F-34094, France, [2]INSERM, U661, Montpellier, F-34094, France, [3]Universités de Montpellier 1 & 2, UMR-5203, Montpellier, F-34094, France, [4]Université Constantine 1, Faculté des sciences de la nature et de la vie. Constantine, Algérie, [5]INSERM U1046, Université Montpellier 1, Université Montpellier 2, Montpellier, France, and [6]Ferring Research Institute Inc, 4245 Sorrento Valley Boulevard, San Diego, CA 92121, USA

BACKGROUND AND PURPOSE
Distinct vasopressin receptors are involved in different physiological and behavioural functions. Presently, no selective agonist is available to specifically elucidate the functional roles of the V_{1A} receptor in the rat, one of the most widely used animal models. FE 201874 is a new derivative of the human selective V_{1A} receptor agonist F180. In this study, we performed a multi-approach pharmacological and functional characterization of FE 201874 to determine whether it is selective for V_{1A} receptors.

EXPERIMENTAL APPROACH
We modified an available human selective V_{1A} receptor agonist (F180) and determined its pharmacological properties in cell lines expressing vasopressin/oxytocin receptors (affinity and coupling to second messenger cascades), in an *ex vivo* model (aorta ring contraction) and *in vivo* in rats (proliferation of adrenal cortex glomerulosa cells and lactation).

KEY RESULTS
FE 201874 exhibited nanomolar affinity for the rat V_{1A} receptor; it was highly selective towards the rat V_{1B} and V_2 vasopressin receptors and behaved as a full V_{1A} agonist in all the pharmacological tests performed. FE 201874 bound to the oxytocin receptor, but with moderate affinity, and behaved as an oxytocin antagonist *in vitro,* but not *in vivo*.

CONCLUSIONS AND IMPLICATIONS
On functional grounds, all the data demonstrate that FE 201874 is the first selective agonist of the rat V_{1A} receptor isoform available. Hence, FE 201874 may have potential as a treatment for the vasodilator-induced hypotension occurring in conditions such as septic shock and could be the most suitable compound for discriminating between the behavioural effects of arginine vasopressin and oxytocin.

Abbreviations
AVP, arginine[8] vasopressin; BrdU, bromodeoxyuridine; InsPs, total inositol phosphates; K_{act}, activation constant for agonists; K_i, inhibitory dissociation constant; K_{inact}, inactivation constant for antagonist; OT, oxytocin; SI, selectivity index

Introduction

Arginine vasopressin (AVP) is a natural neurohypophyseal neuropeptide synthesized in the mammalian hypothalamus that controls various physiological peripheral effects, among which water reabsorption and vascular tone regulation are the most widely reported (for review see Jard, 1998; Koshimizu et al., 2012). AVP in synergism with corticotropin-releasing factor contributes to the regulation of adrenocorticotropic hormone release (Gillies et al., 1982; Abou-Samra et al., 1987), catecholamines (Grazzini et al., 1998), insulin (Lee et al., 1995) and glucagon (Yibchok-Anun et al., 1999) and is involved in the proliferation of adrenal cortex and kidney medullary cells (Alonso et al., 2009). More recently, it was clearly demonstrated that AVP and its sibling hormone oxytocin (OT) are major regulators of brain functions such as the stress response, memory, and affective and social behaviour (De Wied, 1971; Caldwell et al., 2008; Meyer-Lindenberg et al., 2011; Stoop, 2012). Peripheral and central AVP functions are mediated by three distinct receptors known as V_{1A}, V_{1B} and V_2, all belonging to the GPCR family. These GPCRs have different structures of their encoding genes, amino acid sequences and coupling properties to second messenger cascades. They can be selectively targeted on the basis of their pharmacological properties (Barberis et al., 1992; Jard, 1998; Thibonnier, 2004). The OT receptor shares common sequences with the AV receptors (see amino acid sequence alignment in Rodrigo et al., 2007). Particularly relevant are two conserved residues Arg 1.27 and Glu 1. 35 (Ballesteros numbering see Rodrigo et al., 2007) located in the N terminal part and the first transmembrane domain, which are essential for both AVP and OT affinities for their specific receptors (Wooten et al., 2011; Rodrigo et al.). Accordingly, AVP exhibits nanomolar affinity for the OT receptor (See Manning et al., 2012 for review), it is thus important to include the OT receptor in pharmacological studies of the AVP receptor family.

The design of specific agonists and antagonists for the AVP and OT receptors is hampered by three major obstacles: (i) The AVP and OT receptors share a large degree of structural homology. Accordingly, AVP is relatively non-specific and interacts with all four AVP/OT receptors with nanomolar affinity (Barberis et al., 1992; Pena et al., 2007). As a consequence, few compounds derived from this endogenous peptide are selective for the V_{1A} receptor. (ii) The pharmacological differences between rat, human and mouse AVP/OT receptor isoforms prevents the discovery of universally selective compounds (Guillon et al., 2006). (iii) The pharmacological profile of a given compound may also depend on the assay used to determine its specificity. On the basis of in vivo tests, F180 may be considered as a selective V_{1A}/V_2 agonist. It exhibits a significant pressor activity (44% of that of AVP) but very low antidiuretic effects (less than 1% of those of AVP). Yet, this functional selectivity is not observed when using binding assays. The affinity of F180 for the rat V_{1A} and V_2 receptors is very low (480 nM and 2000 nM respectively) as compared to the nanomolar affinity of AVP for these same receptors (Andrès et al., 2002). Similarly, [Phe^2]-[2-phenylalanine-8-ornithine]-vasotocin behaves as a selective vasopressor peptide in vivo, but exhibits no V_{1A} binding selectivity (Manning et al., 2012 and unpublished results).

A recent review from M. Manning and our group highlights the lack of a selective rat V_{1A} receptor agonist (Manning et al., 2012). Even though F180 and numerous recently described derivatives display high affinity and selectivity for the human V_{1A} receptor and have potential therapeutic use, primarily in the treatment of vasodilator-associated hypotension such as in septic shock, they display no specificity when tested in vitro on the rat vasopressin receptors (Andrès et al., 2002; Wisniewski et al., 2011). As recent studies confirm that AVP and OT are major central regulators of many behavioural functions in mammals (for review see Caldwell et al., 2008; Koshimizu et al., 2012) and are also involved in functions in many peripheral tissues, in particular the cardiovascular system (Gutkowska and Jankowski, 2012) and in obesity (Deblon et al., 2011), it is of major interest to determine which receptor isoform is responsible for which effect. Rat and mouse are the species most widely used in animal models. Selective rodent OT and V_{1B} receptor agonists are available but the design of selective V_{1A} receptor agonists appears to be essential to complete the arsenal of pharmacological tools available to better understand how endogenous neuropeptides like AVP regulate CNS functions. Indeed F180, described as the first selective agonist for the human V_{1A} receptor, does not discriminate between the rat AVP/OT receptors (Andrès et al., 2002).

In the present study, we characterized the pharmacological properties of a new derivative FE 201874, and demonstrated, using multiple in vitro, ex vivo and in vivo approaches, that this peptide can be considered to be the first selective agonist of the rat V_{1A} receptor isoform available.

Methods

The nomenclature used for drug targets conforms to the BJP's Guide to Receptors and Channels (Alexander et al., 2011).

Cell culture, stable transfection and membrane preparation

HEK cells expressing rat V_2 receptor (rV_2 receptor) or mouse oxytocin receptor (mOT receptor) and CHO cells stably expressing human V_{1A} receptor (hV_{1A} receptor), human V_{1B} receptor, human V_2 receptor, human oxytocin receptor, or rat oxytocin receptor were maintained in culture in DMEM; AtT20 (pituitary adenoma) cells expressing rV_{1B} receptors or mV_{1B} receptors were cultured in DMEM/F12 as previously described (Pena et al., 2007); and WRK1 (rat mammary tumour) cells naturally expressing the rV_{1A} receptor were grown as previously described (Kirk et al., 1986). In some experiments, cells were treated overnight with 5 mM sodium butyrate to increase receptor expression (Kassis et al., 1984). Membranes from CHO and AtT20 cells were prepared according to Murat et al. (2012). Membranes from liver, kidney and anterior pituitary were obtained as described previously (Andrès et al., 2002) from Wistar rats or adult C57BL/6 mice.

Binding assays

Membrane incubations with [^{3}H]-AVP were performed as described previously (Andrès et al., 2002). For saturation binding experiments, 5–20 µg membrane protein were incubated 60 min at 30°C (membranes from CHO, AtT20 or

WRK1 cells) or 37°C (membranes from native tissues) in 200μl of a medium containing: 50 mM Tris-HCl (pH 7.4), 3 mM MgCl$_2$, 1 mg·mL^{-1} BSA, 0.01 mg·mL^{-1} leupeptine and increasing concentrations (0.5 to 7 nM) of [^{3}H]-AVP with (non-specific binding) or without (total binding) 1 μM of unlabelled AVP or OT. For competition experiments, 5–20 μg membrane protein were incubated as described above with 1 nM [^{3}H]-AVP and increasing amounts of unlabelled FE 201874. Plasma membrane-associated radioactivity was determined by filtration through GF/C filters and specific binding calculated in each condition as the difference between total and non-specific binding.

Inositol phosphate assays

Inositol phosphate (InsPs) accumulation was determined as described previously (Derick et al., 2002). Briefly, CHO or AtT20 cells stably transfected with rOT or rV$_{1B}$ receptors, respectively, or WRK1 cells that naturally expressed the rV$_{1A}$ receptor were plated at 100 000 cells per well. Cells were grown for 24 h in their respective culture medium (see above) and further incubated for another 24 h in a serum- and inositol-free medium supplemented with 2 μCi·mL^{-1} myo-[2-^{3}H]-inositol. Cells were then washed twice with Hank's buffered saline, incubated for 15 min in 20 mM LiCl, and further stimulated for 15 min with increasing concentrations of analogues to be tested. The reaction was stopped by addition of perchloric acid (5% v v^{-1}). Total accumulated InsPs were extracted and purified on Dowex AGI-X8 anion exchange chromatography column as described previously and counted.

Adenylyl cyclase assays

HEK cells expressing rV$_2$ receptors were grown as described above, and the production of cAMP assessed after 2 days in culture as previously described (Murat et al., 2012). Cells were incubated in [^{3}H]-adenine (3 μCi·mL^{-1}) for 24 h and then with DMEM supplemented with 5.5 mM IBMX and 0.1% BSA with the vehicle alone or various concentrations of agonists for 10 min at 37°C. Intracellular cAMP concentrations were determined by measuring [^{3}H]-cAMP and expressed as % of the sum of radioactivity recovered in the cAMP fraction and radioactivity that was not retained by the Dowex column, which mainly corresponded to labelled ATP.

Animal care, animal treatments

Adult male Sprague Dawley rats (200–250 g) and adult C57BL/6 mice were purchased from Janvier (Le Genest-St-Isle, France). They were housed in light- (12 h dark and 12 h light) and temperature- (21°C) controlled rooms with free access to standard dry food and tap water. All animals were treated in accordance with the principles of laboratory animal care of the ARRIVE guidelines (McGrath et al., 2010; Kilkenny et al., 2011) and those published by the French Ethical Committee and under the supervision of an authorized investigator.

All procedures in this study conformed to the animal welfare guidelines of the European Community and were approved by the local ethical committee (authorizations n°34.128 for experimentation, n° IGF-2012-001A for this specific protocol).

Vascular reactivity measurements

Vasopressin analogues were tested on isolated vascular rings obtained from rat aorta as described previously (Cordaillat

et al., 2007). Aortic segments were subjected to a 60 min equilibration period at the predetermined optimal point of the active length-tension curve previously established at 2 g for rat aorta. The contractile function of each segment was assessed with 1 μM phenylephrine. After a wash-out and a stabilization period of 20 min, responses to cumulative concentrations of AVP, FE 201874, d[Leu^4,Lys^8]VP and dDAVP were determined. At the end of the dose-response, a saturating concentration of AVP (100 nM) was applied to normalize contraction to the maximal AVP-induced response. Changes in isometric tension were recorded using an IT1-25 transducer and an IOX computerized system (EMKA Technologies, Paris, France).

Proliferation measurements

Cell proliferation under administration of vasopressin and/or compounds to be tested was determined in vivo as previously described (Alonso et al., 2009). Rats were anaesthetized by inhalation of isoflurane (1.5% in O$_2$) and implanted s.c. with an Alzet 7 days osmotic pump filled with AVP, dDAVP, d[Leu^4, Lys^8]VP, [Thr^4,Gly^7]OT or FE 201874 at different concentrations or just operated on without any pump implantation (sham). Some rats were treated twice-daily for 3 days with i.p. injections of saline or of a specific antagonist of the rV$_{1A}$ receptor (SR49059; at 1 mg·kg^{-1}). Because this antagonist is poorly water soluble, it was dissolved in saline containing 5% DMSO + 7.5% Cremophor (v v^{-1}).

At the end of the infusion of the various agonists, rats received two i.p. injections of bromodeoxyuridine (BrdU) (100 mg·kg^{-1} in 0.5 ml 0.01 N NaOH) 16 and 5 h before fixation. Under deep pentobarbital anaesthesia (50 mg·kg^{-1} i.p.), animals were perfused through the ascending aorta with PBS (pH 7.4) followed by 400 mL paraformaldehyde (4% in 0.1 M phosphate buffer; pH 7). The adrenal gland, liver, kidney and pituitary were dissected out and fixed overnight in the same fixative. Sections (50 μm) were then cut with a vibratome (Leica VT1000S) incubated in 2N HCl overnight at 4°C and for 48 h at 4°C with a mouse monoclonal anti-BrdU antibody. Sections were then incubated for 4 h at room temperature with a donkey anti-mouse monoclonal antibody conjugated with Cy3 (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). The primary and secondary antibodies were diluted in PBS containing 0.1% Triton X-100.

Labelled sections were mounted in Mowiol, and observed under a Zeiss LSM510 Meta confocal microscope using x10 or x20 objectives (excitation at 550 nm; emission at 568 nm). The background noise was reduced by averaging four image inputs. Digitalized images were analysed with Image J (Adobe, San Jose, CA, USA). For this, we counted the labelled nuclei detected in the adrenal cortex area of each section, with four sections per animal and four animals per experimental group.

Measurements of pup growth

In order to determine putative antagonistic properties of FE 201874 on the OT receptor, rat dam lactation was evaluated by following pups growth. Lactating dams were delivered to the laboratory on the second day after pups' birth. The number of pups was reduced to 8 per dam and pups were then weighed individually twice daily (at 0900 and 1700 h).

On the seventh day of lactation, dams were injected i.p. twice (at 0900 and 1400 h) with either vehicle or the pharmacological compound (in 0.5 ml) to be tested. The weights of 24–32 pups from 3 to 4 dams were averaged in each condition. FE 201874, SSR126768A and SR49059 were first diluted in pure DMSO, pure Cremophor was further added to provide better bioavaibility (Alonso *et al.*, 2009) and the volume was complemented with sterile NaCl 0.9% to reach final concentrations of 5% DMSO and 7.5% Cremophor. Results are expressed as mean ± SEM. Student's unpaired *t*-test was used to compare means.

Data analysis

The radioligand binding, InsP and cAMP accumulation, vascular reactivity measurements and proliferation measurements data were analysed by GraphPadPrismTM (GraphPad software, Inc, San Diego, CA, USA). K_d values were deduced from Scatchard experiments and K_i from competition experiments as previously described. The inhibitory dissociation constants (K_i) for unlabelled AVP analogues were calculated from binding competition experiments according to the Cheng and Prusoff (1973) equation: $K_i = IC_{50}(1 + [L]/K_d)$, where IC_{50} is the concentration of unlabelled analogue leading to half maximal inhibition of specific binding, [L] the concentration of the radioligand present in the assay and K_d its affinity for the AVP receptor studied. K_{act} and K_{inact} values were calculated from functional studies. K_{inact} was calculated from IC_{50} using the following equation: $K_{inact} = IC_{50}(1 + [H]/K_{act})$, where [H] is the concentration of agonist used in the assay (Andrès *et al.*, 2002). Results are expressed as the mean ± SEM of at least three distinct experiments.

Results

Chemical structure of FE 201874

FE 201874 was synthesized as previously described (Wisniewski *et al.*, 2011). It is an analogue of AVP in which Tyr^2 was substituted by Phe, Phe^3 by Ile, Gln^4 by Hgn and Arg^8 by Orn. As compared to the F180 molecule discovered earlier (Aurell *et al.*, 1991) and described as a selective agonist for human V$_{1A}$ receptors (Andrès *et al.*, 2002), FE 201874 contains two modifications: Hmp^1 ((R)-2-hydroxy-3-mercaptopropionic acid) is replaced by Cys and Dab(Abu)^8 ((S)-2-amino-4-((S)-2-aminobutyryl)aminobutyric acid) is replaced by Orn (see Figure 1).

Binding properties of FE 201874

The affinity of FE 201874 for various AVP/OT receptor isoforms from various mammalian species was determined by classical binding competition experiments on membrane preparations using [^{3}H]-AVP (Table 1). FE 201874 dose-dependently inhibited [^{3}H]-AVP binding ($K_i = 7.4$ nM; Table 1) on plasma membranes derived from a rat mammary tumour cell line (WRK1) expressing the rV$_{1A}$ receptor. When similar experiments were performed on crude plasma membranes derived from rat tissues naturally expressing the native rV$_2$ or rV$_{1B}$ receptor subtypes (kidney or pituitary gland respectively) or on plasma membranes derived from AtT20 and CHO cells stably transfected with rV$_{1B}$ or rOT receptor,

respectively, FE 201874 was less effective at displacing [^{3}H]-AVP (Table 1). The K_i value of FE 201874 on crude rat liver plasma membranes naturally expressing rV$_{1A}$ receptors was also in the nanomolar range (Figure 2, Table 1). Binding selectivity indexes (SI) were calculated as described previously (Andrès *et al.*, 2002). With rat receptors, whatever the membrane preparation tested (from WRK1 cells or liver tissue), FE 201874 displayed a SI of 427 and 29 for V$_2$/V$_{1A}$ receptor and V$_{1B}$/V$_{1A}$ receptor, respectively (see Table 1), indicative of a specific binding to V$_{1A}$ receptors among the AVP receptors. However, FE 201874 also displayed a medium selectivity towards the OT receptor (OT/V$_{1A}$ receptor SI = 6). Similar results were obtained with the human V$_{1A}$ receptor for which FE 201874 exhibited a nanomolar affinity as well as high V$_{1B}$/V$_{1A}$ and V$_2$/V$_{1A}$ receptor selectivity and a medium one for OT/V$_{1A}$ receptor (Figure 2B and Table 1). However, as shown in Table 1, FE 201874 exhibited a higher affinity for the mOT receptor ($K_i = 20$ nM), than for the mV$_{1A}$ receptor ($K_i = 40.1$ nM), intermediate affinity for the mV$_{1B}$ receptor ($K_i = 184$ nM) and a much lower affinity for the mV$_2$ receptor ($K_i = 597$ nM). As a consequence, FE 201874 is not selective for the mouse V$_{1A}$ receptor.

Functional properties of FE 201874

To pursue the pharmacological characterization of FE 201874, we analysed production of the signal transduction and intracellular messengers (InsP and cAMP) on cell lines expressing AVP/OT receptors. On WRK1 cells stably expressing rV$_{1A}$ receptors, FE 201874 dose-dependently stimulated InsP accumulation with a maximal efficacy representing 85% of that obtained with AVP and a K_{act} of 7.7 nM (Figure 3A and Table 2). Pre-incubation with increasing concentrations of SR49059, a selective V$_{1A}$ receptor antagonist (Serradeil-Le Gal *et al.*, 1993), dose-dependently inhibited FE 201874-stimulated InsP accumulation (Figure 3B). The K_i of SR49059 was 2.2 ± 0.4 nM (three experiments), a value very close to the affinity of SR49059 for the rV$_{1A}$ receptor (Serradeil-Le Gal *et al.*, 1993).

To determine the functional selectivity of FE 201874, we further examined its pharmacological properties on cell lines expressing rV$_{1B}$ receptors (AtT20), rV$_2$ receptors (CHO) or rOT receptors (CHO). As illustrated in Figure 4A and B, FE 201874 displayed full agonist properties rV$_{1B}$ and rV$_2$ receptors, since it stimulated InsP and cAMP accumulation as effectively as AVP (Table 2). However, the K_{act} of FE 201874 for these two receptors was much higher than that for rV$_{1A}$ receptors (Table 2). By contrast FE 201874, even tested at 1 μM, only very weakly stimulated rOT receptor-mediated InsP accumulation (6 ± 3% of maximal OT stimulation). More interestingly, increasing concentrations of FE 201874 added to 5 nM OT completely inhibited OT-stimulated InsP accumulation (Figure 4D), with a K_i of 40.1 ± 7.8 nM (three experiments). These data indicate that, besides V$_{1A}$ agonism, FE 201874 behaves as a potent rat OT antagonist *in vitro*.

Effects of FE 201874 on rat arterial contraction

We have previously shown (Ryckwaert *et al.*, 2009) that AVP can induce a sustained contraction of rat aorta. Thus, this model appears appropriate to functionally characterize the

AVP
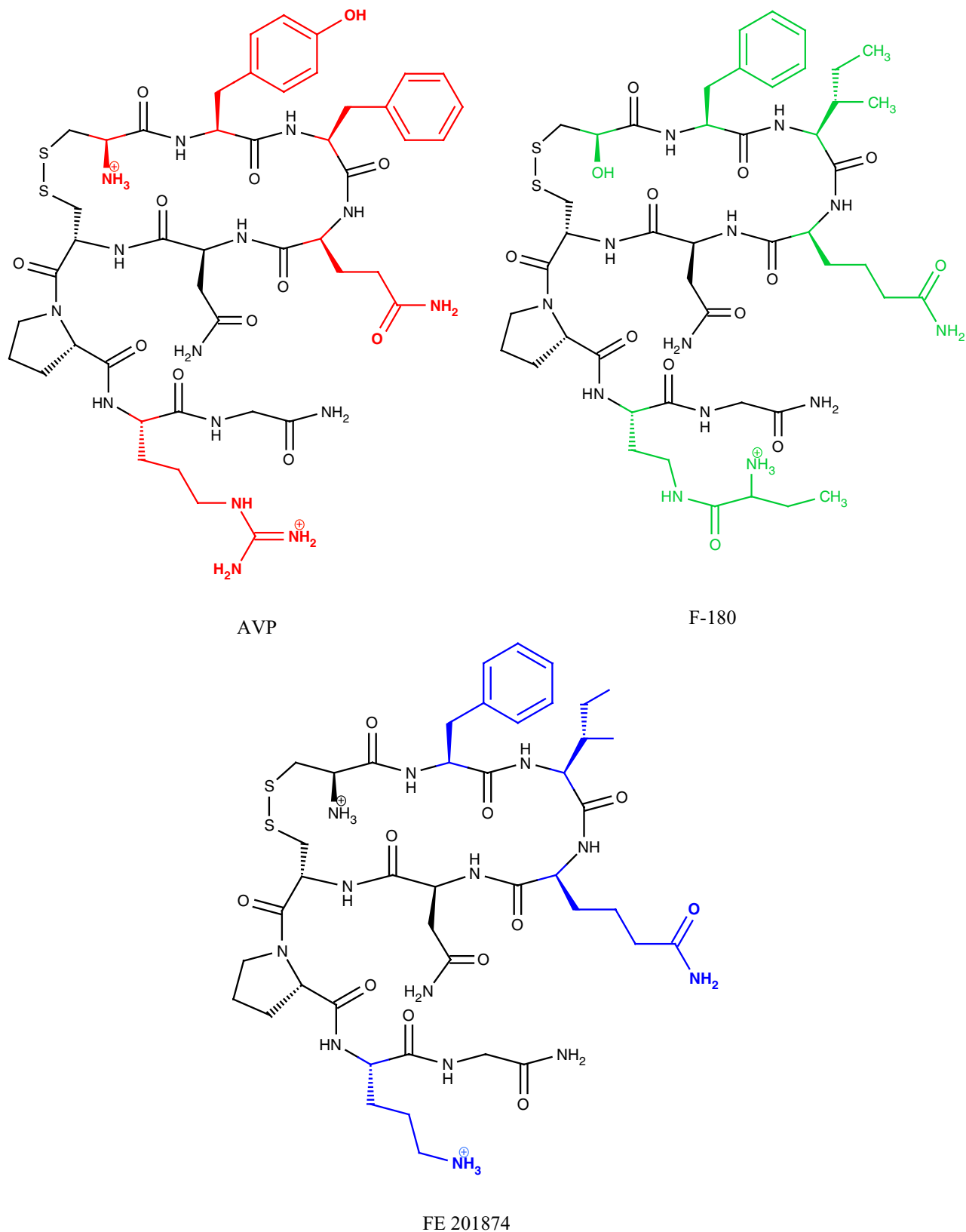
F-180

FE 201874

Figure 1

Molecular structures of AVP, F180 and FE 201874. The structures are shown as fully protonated forms. F180 and FE 201874 are analogues of AVP. Red colour indicates the modifications applied in AVP to design F180 and FE 201874. Green colour indicates the differences between F180 and AVP and blue colour indicates the differences between FE 201874 and AVP.

Table 1

Affinity of FE 201874 for AVP/OT receptors from different mammalian species

	Receptor		K_d or K_i (nM)			
Species	Isoform	Source	AVP[a]	FE 201874	V_{1A}-S.I.	
Rat	V_{1A}	WRK1 cells	0.65 ± 0.04	7.4 ± 1.4	1.0	
		Liver	1.5 ± 0.1	9.6 ± 0.7		1.0
	V_{1B}	AtT20 cells	0.49 ± 0.06	652 ± 113	117	
		Pituitary	3.4 ± 0.4	626 ± 103		28.8
	V_2	Kidney	0.72 ± 0.11	1966 ± 228		427
	OT	CHO cells	0.9 ± 0.2	61.8 ± 14.2	6.0	
Human	V_{1A}	CHO cells	1.1 ± 0.1	7.4 ± 1.1	1.0	
	V_{1B}	CHO cells	0.68 ± 0.01	676 ± 146	148	
	V_2	CHO cells	1.2 ± 0.2	$>10\,000$	>1239	
	OT	CHO cells	1.7 ± 0.5	89.2 ± 1.3	7.8	
Mouse	V_{1A}	Liver	2.1 ± 0.4	40.1 ± 2.2	1.0	
	V_{1B}	AtT20 cells	0.57 ± 0.03	184 ± 8	16.9	
	V_2	Kidney	0.21 ± 0.04	597 ± 109	149	
	OT	HEK293 cells	2.3 ± 0.3	20.0 ± 1.2	0.5	

Affinity (K_d) for [³H]-AVP (nM) was determined from saturation binding experiments performed on plasma membranes derived from cell lines stably expressing the different AVP/OT receptors or from tissue extracts as described in the experimental section. K_i values for FE 201874 were deduced from competition binding experiments illustrated in Figure 2 and from unshown results and are the mean $\pm$ SEM of at least three independent experiments, each performed in triplicate. For each analogue, the rat V_{1A} selectivity Index (V_{1A}-S.I.) was calculated as follows: SI = (K_i analogue for Vx receptor/K_d AVP for Vx receptor)/(K_i analogue for rV_{1A} receptor/K_d AVP for V_{1A} receptor), where Vx receptor is the V_{1B}, V_2 or OT receptor. For the rat receptor, SI were calculated using as reference binding data obtained on native tissue (left column) or on cultured cells (right column).
[a](Pena et al., 2007).

Table 2

Pharmacological and physiological properties of FE 201874 for rat AVP/OT receptors

Receptors				Peptide		
Isoform	Source	Functional parameters	AVP	OT	FE 201874	
rV_{1A}	Rat aorta ring	K_{act} (nM)	7.9 ± 1.3	nd	49.6 ± 3.8	
		E_{max} (%)	100		94 ± 4	
	Adrenal cortex	K_{act} ($\mu g \cdot kg^{-1}$)	0.89 ± 0.04	nd	1.4 ± 0.1	
		E_{max} (%)	100		81.3 ± 5.4	
	WRK1 cells	K_{act} (nM)	0.59 ± 0.16	nd	7.7 ± 1.0	
		E_{max} (%)	100		85.2 ± 3.3	
rV_{1B}	AtT20 cells	K_{act} (nM)	2.3 ± 0.8	nd	140.3 ± 31.7	
		E_{max} (%)	100		83.5 ± 1.3	
rV_2	HEK293 cells	K_{act} (nM)	0.19 ± 0.12	nd	938 ± 193	
		E_{max} (%)	100		97.7 ± 6.9	
rOT	CHO cells	K_{act} / K_{inact}[I] (nM)	nd	2.26 ± 0.33	40.1 ± 7.8[I]	
		E_{max} (%)		100	6.7 ± 0.7	

Pharmacological (measurements of second messengers accumulation) and physiological (measurement of rat aorta contraction and rat zona glomerulosa cell proliferation) assays were performed as described in legends of Figures 3 to 6 and in Methods. K_{act} or K_{inact} were deduced from dose-response curves as illustrated on Figures 3–5 as previously described (Pena et al., 2007). For each assays, maximal efficacy was calculated as the percentage of the maximal response obtained with 100 nM AVP (V_{1A}, V_{1B} and V_2 receptor) or 100nM OT (OT receptor). Results are the mean $\pm$ SEM of at least three distinct determinations each performed in triplicate.
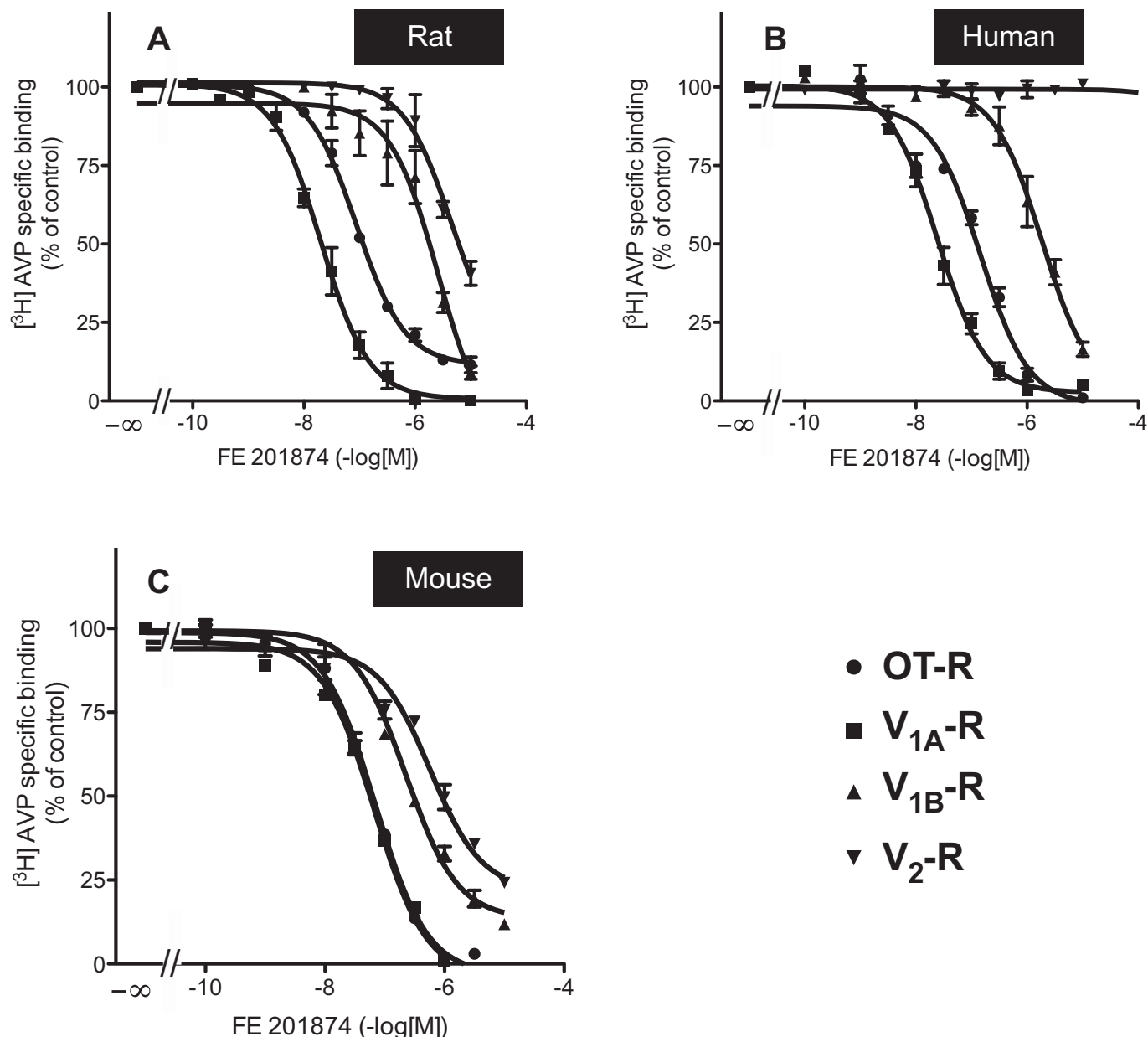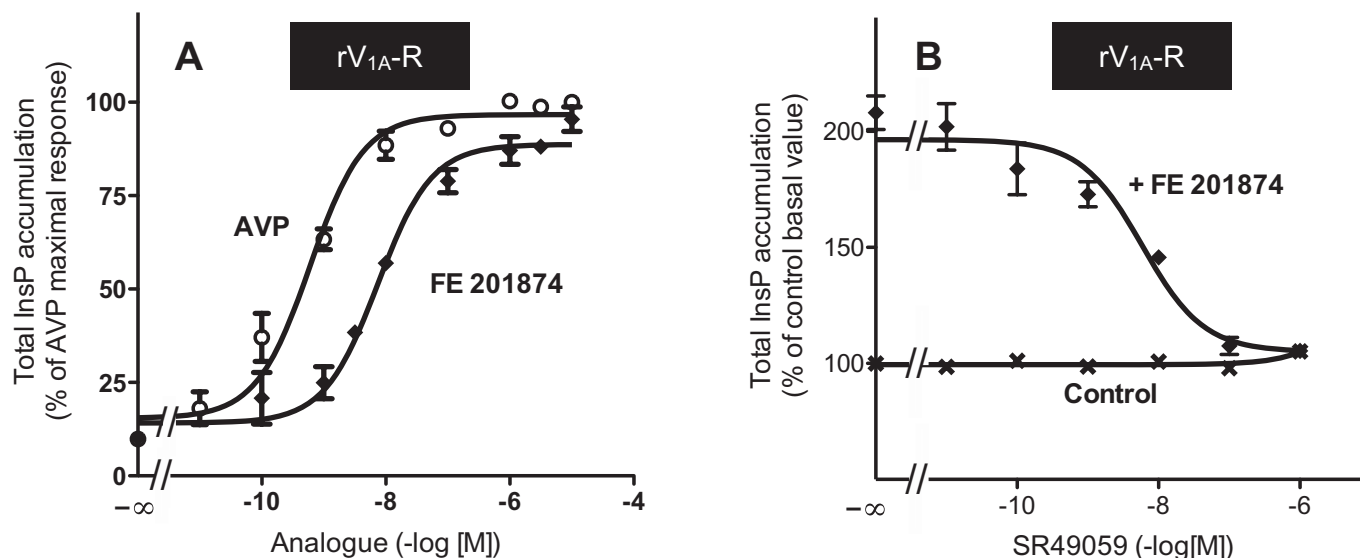
Figure 2

Binding properties of FE 201874 for mammalian vasopressin and oxytocin receptors. Plasma membranes obtained from stably transfected CHO cells expressing rOT receptors, AtT20 cells expressing rV_{1B} receptors , WRK1 cells and rat liver naturally expressing rV_{1A} receptors and rat kidney naturally expressing rV_2 receptors (A), from CHO cells stably expressing hAVP/OT-Rs (B) and from HEK293 cells transfected transitively with mOT receptors , AtT20 cells expressing mV_{1B} receptors, mouse liver naturally expressing mV_{1A} receptors and mouse kidney naturally expressing mV_2 receptors (C), were incubated with 1 nM [³H]AVP in the presence or absence (control) of increasing amounts of FE 201874. Specific binding was calculated for each condition and expressed as % of corresponding control values. Results are the mean ± SEM of at least three distinct experiments each performed in triplicate.

agonist properties of FE 201874. We observed that this derivative induced a sustained and dose-dependent contraction of rat aorta (Figure 5A and C), which developed within a few seconds after application of 100 nM FE 201874. Moreover, the maximal contraction induced (2.1 ± 0.3 g) is identical to that obtained with AVP (2.2 ± 0.2 g) (Figure 5B). In the same conditions, [Thr⁴,Gly⁷]OT and dDAVP did not induce

contraction (Figure 5C). d[Leu⁴,Lys⁸]VP was less effective than FE 201874 with a higher K_{act} (Table 2) and a maximal effect representing 37 ± 8 % of that of FE 201874. As expected, increasing concentrations of the selective rV_{1A} receptor antagonist SR49059 completely antagonized the effect of a non-maximal concentration of FE 201874 (10nM) with a K_i of 1.8 ± 0.2 nM (three experiments).

Figure 3

Agonist properties of FE 201874 on rat V$_{1A}$ receptors. (A) myo-[2,^{3}H]-inositol prelabelled WRK1 cells naturally expressing the rV$_{1A}$ receptor were pre-incubated for 15 min at 37°C with 20 mM LiCl and further stimulated for 15 min with or without (control) increasing concentrations of AVP or FE 201874. Total InsPs accumulated are expressed as percentage of maximal AVP response. (B) myo-[2,^{3}H]-inositol prelabelled cells were pre-incubated for 15 min at 37°C with 20 mM LiCl and increasing concentrations of SR49059 or vehicle (control). Then, 10 nM FE 201874 or vehicle was added in the incubation medium and the reaction allowed to proceed for another 15 min period. InsPs, which accumulated, were measured and expressed as percentage of InsPs accumulated under control FE 201874 stimulation. Values are given as the mean ± SEM of two or three independent experiments each performed in triplicate.

Proliferative effect of FE 201874 in the rat adrenal gland

Vasopressin, through the V$_{1A}$ receptor, has been shown to be involved in adrenal glomerulosa cell proliferation (Grazzini et al., 1998; Alonso et al., 2009) (see also Figure 6A). To validate this in vivo model, we first verified that selective V$_{1B}$ (d[Leu^4,Lys^8]VP), V$_2$ (dDAVP) and OT ([Thr^4,Gly^7]OT) receptor agonists were unable to induce proliferation of rat glomerulosa cells even when tested at 128 µg·day^{-1} for 3 days (Figure 6B). These data confirm that the adrenal cortex expresses only functional V$_{1A}$ receptors involved in cell proliferation and thus represents a convenient in vivo model for testing selective V$_{1A}$ agonists.

Rats were then infused for 3 days with increasing doses of FE 201874 or AVP (from 0.04 to 13 µg·day^{-1}) or vehicle (control). As compared to controls, the adrenal cortex of rats infused with FE 201874 contained numerous BrdU positive cells mostly localized in the zona glomerulosa (Figure 6A). This effect was saturable, dose-dependent with a K$_{act}$ of 1.4 ± 0.1 µg·day^{-1} (three experiments), and comparable to that induced by AVP but with a slightly lower K$_{act}$ (see Table 2). When SR49059 was injected twice daily (2.5 mg·day^{-1}; i.p.) to FE 201874-infused rats, the proliferative effect of FE 201874 was completely abolished (Figure 6A), thus demonstrating that FE 201874-induced proliferation was mediated entirely by activation of V$_{1A}$ receptors.

Control experiments also showed that FE 201874, even when tested at 60 µg·day^{-1}, did not induce rat tubular cell proliferation as observed with dDAVP, a specific V$_2$ agonist (Alonso et al., 2009). Similarly, FE 201874 did not modify rat pituitary cell proliferation under the same experimental conditions (data not shown).

Putative OT antagonist properties of FE 201874 tested in vivo

Binding and functional studies indicated that FE 201874 has a moderate affinity for the OT receptor and displays antagonist properties. It is thus possible that this compound also displays antagonistic OT properties when injected into living animals. To test for this putative antagonistic action, we injected FE 201874 to lactating rats and monitored lactation efficiency by measuring the pups' weight. In control dams, injection of NaCl did not affect the pups' growth curve (Figure 7A). On the other hand, injection of the oxytocin receptor antagonist SSR126788A (2.5 mg·day^{-1}) (Serradeil-Le Gal et al., 2004) induced an expected transient break (24 hours delay) in the growth curve, confirming that an oxytocin antagonist prevents normal lactation. Growth was then restored with a slightly accelerated rate so that the difference between pups from control and injected dams progressively became non-significant. Injection of FE 201874 (0.12 mg·day^{-1}) induced a larger break and a maintained shift in the growth rate (Figure 7B). This effect was completely prevented by a pre-injection of the V$_{1A}$ antagonist SR49059 (6 mg·day^{-1}), demonstrating that it resulted mostly from activation of the V$_{1A}$ receptor.
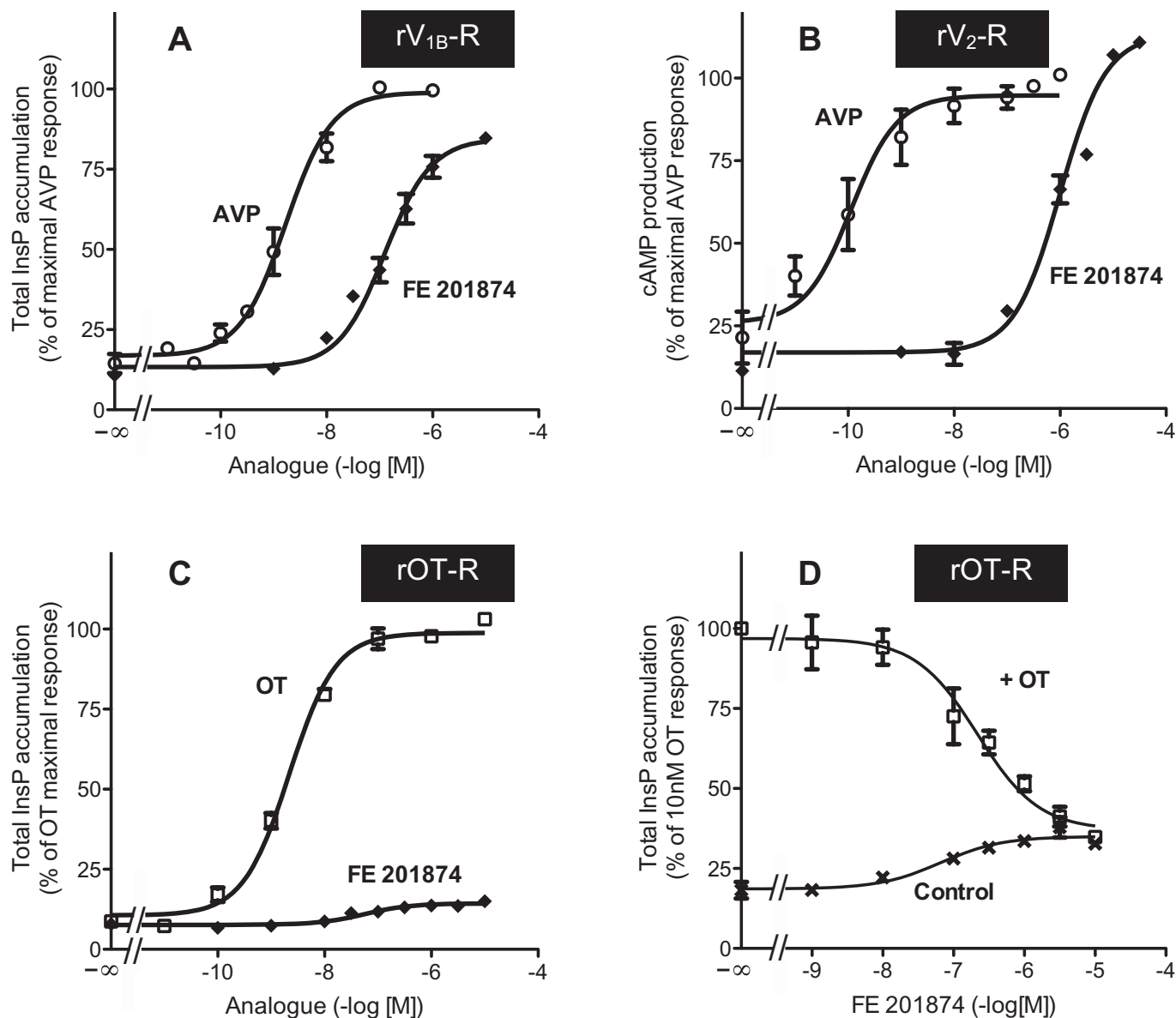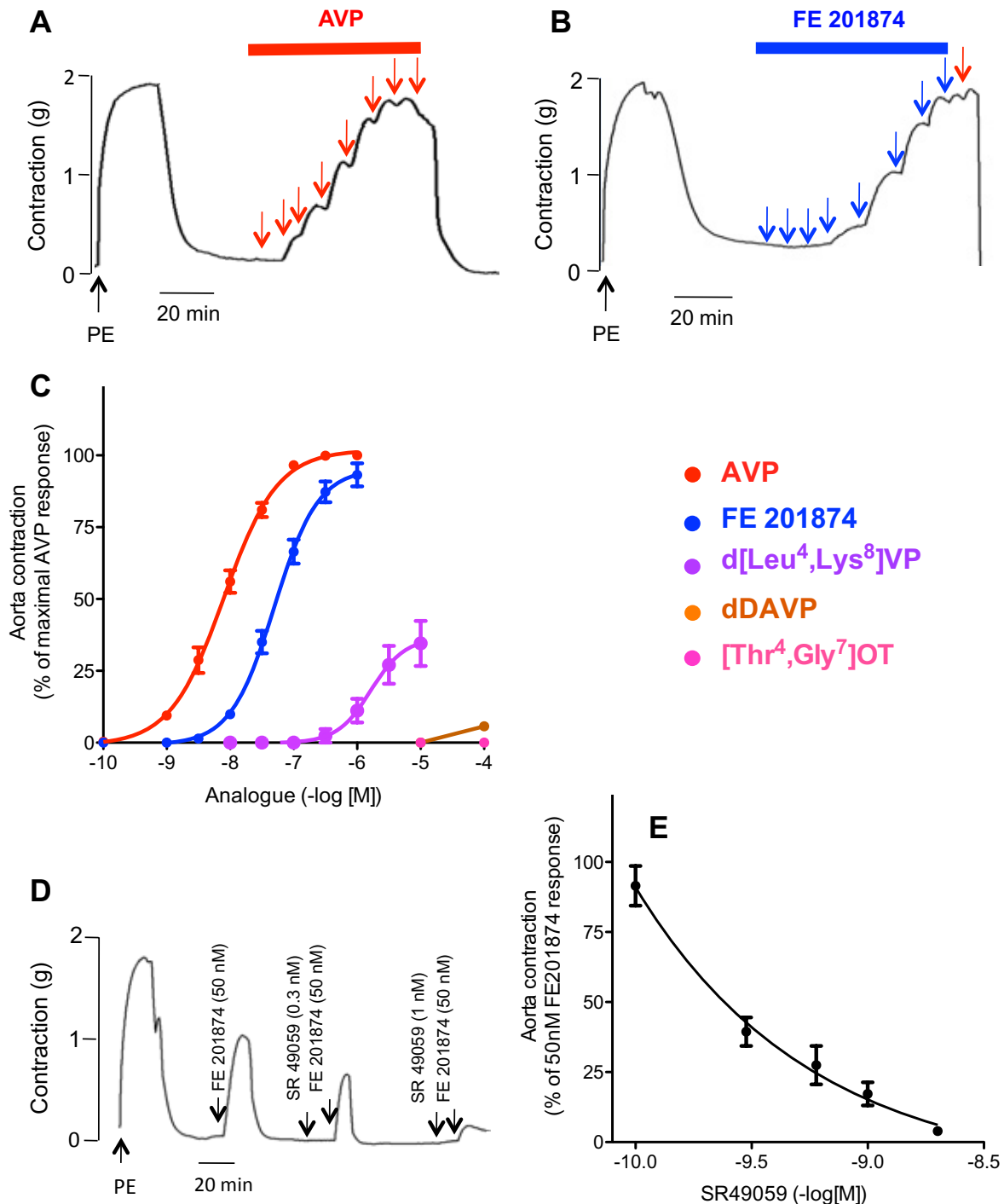
Figure 4
Agonist/antagonist properties of FE 201874 on rat V_{1B}, V_2 and OT receptors. (A) myo-[2,^{3}H]-inositol prelabelled AtT20 cells stably transfected with DNA encoding the rV_{1B} receptor were pre-incubated for 15 min at 37°C with 20 mM LiCl and further stimulated with increasing concentrations of AVP or FE 201874. Total InsP, which accumulated, are expressed as percentage of maximal AVP response. (B) [^{3}H]-adenine prelabelled HEK293 cells transitively transfected with DNA encoding the rV_2 receptor were incubated with or without (control) increasing concentrations of AVP or FE 201874. cAMP, which accumulated, is expressed as % of maximal AVP response. (C) myo-[2,^{3}H]-inositol prelabelled CHO cells stably transfected with DNA encoding the rOT receptor were pre-incubated for 15 min at 37°C with 20 mM LiCl and further stimulated with increasing concentrations of OT or FE 201874. Total InsPs, which accumulated, are expressed as percentage of maximal OT responses. (D) myo-[2,^{3}H]-inositol prelabelled cells expressing the rOT receptor were pre-incubated for 15 min at 37°C with 20 mM LiCl with or without increasing concentrations of FE 201874. Then, 10 nM OT or vehicle (control) was added in the incubation medium and the reaction allowed to proceed for another 15 min period. InsPs, which accumulated in each experimental condition, was measured and expressed as percentage of InsPs accumulated under OT stimulation in the absence of FE 201874. Each point represents the mean ± SEM of three independent experiments, each performed in triplicate.

Discussion

In this study, we describe the first selective V_{1A} agonist for the rat species and have characterized its pharmacological and physiological properties. This compound will complement F180, a V_{1A} receptor agonist specific for human and bovine receptors (Andrès *et al.*, 2002). It will also complete the battery of available tools to elucidate the respective contribution of AVP/OT receptors in physiological and cognitive processes.

Figure 5

Vasoconstriction properties of FE 201874 on rat aorta. Isometric tensions (g) of aortic rings were recorded according to the following protocols. Phenylephrine 1μM (PE) was first added to the incubation medium to control the viability of each aortic ring. After a wash-out and a stabilization period, increasing amounts of AVP (A) or FE 201874 (B) were added to reach the final concentrations of 0.1, 1, 3.16, 10, 31.6, 100, 316, 1000 nM. Vertical arrows indicate the times of application. A final concentration of 100 nM AVP was added at the end of each cumulative dose-response to normalize contraction to the maximal response to AVP. (C) Summarizes dose-response curves for AVP, FE 201874, d[Leu^4,Lys^8]VP, [Thr^4,Gly^7]OT and dDAVP deduced from traces illustrated in (A and B) and from unshown results. Data, expressed as percentage of the maximal contraction induced by 100 nM AVP, are the mean ± SEM of at least five distinct experiments. (D) Effect of a V_{1A} antagonist on FE 201874 contractile response. Aortic rings were pre-incubated or not for 10 min with increasing amounts of SR49059 (from 0.1 to 2 nM) before addition of a non-maximal concentration of FE 201874 (50 nM). Vertical arrows indicate the times of application of each compound. (E) Dose-response curve of the inhibitory effect of SR49059 deduced from traces illustrated in (D). Data are expressed as percentage of the contraction induced by 50 nM FE 201874 in the absence of SR49059 and represent the mean ± SEM of at least five experiments.

A

B
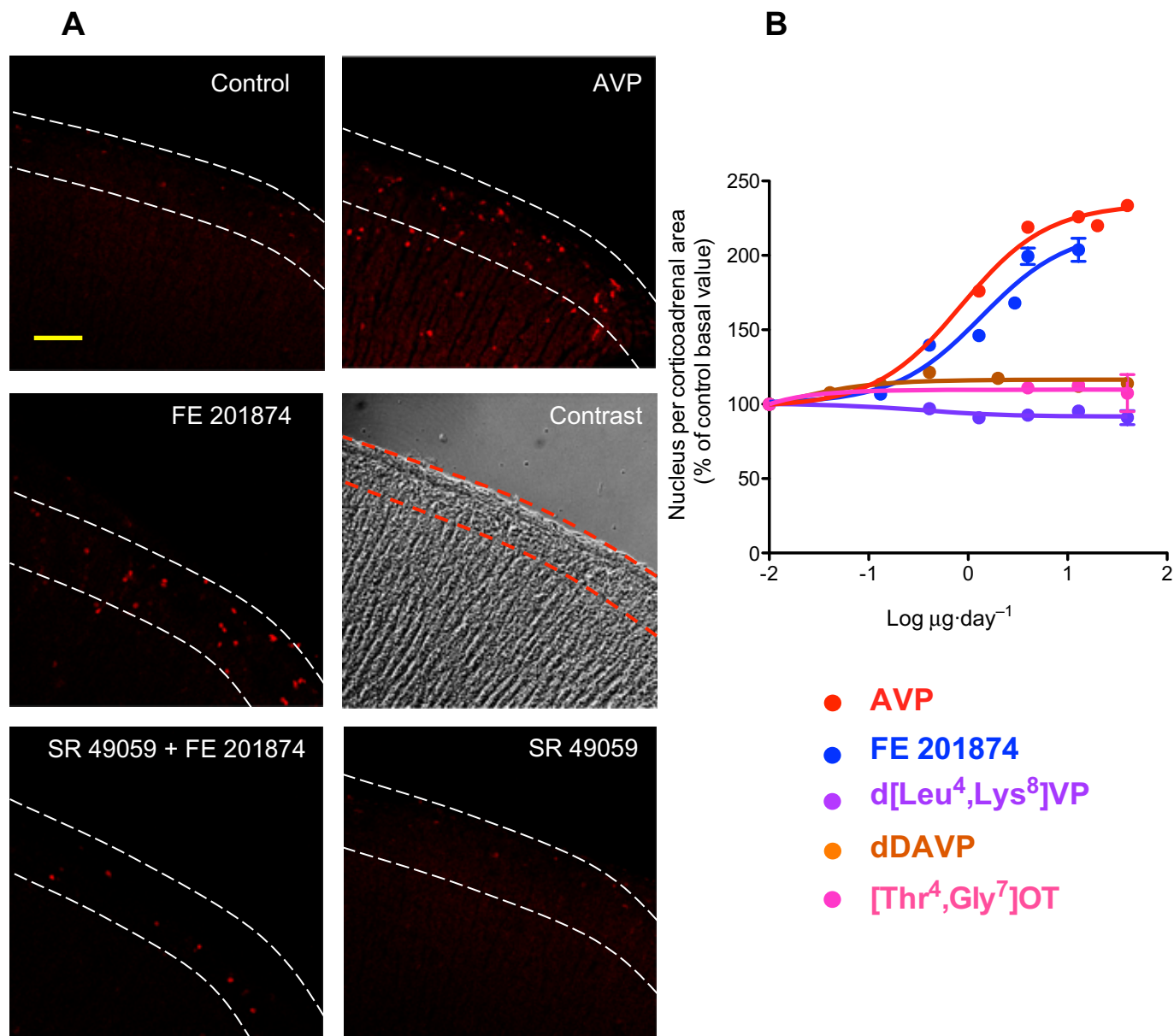
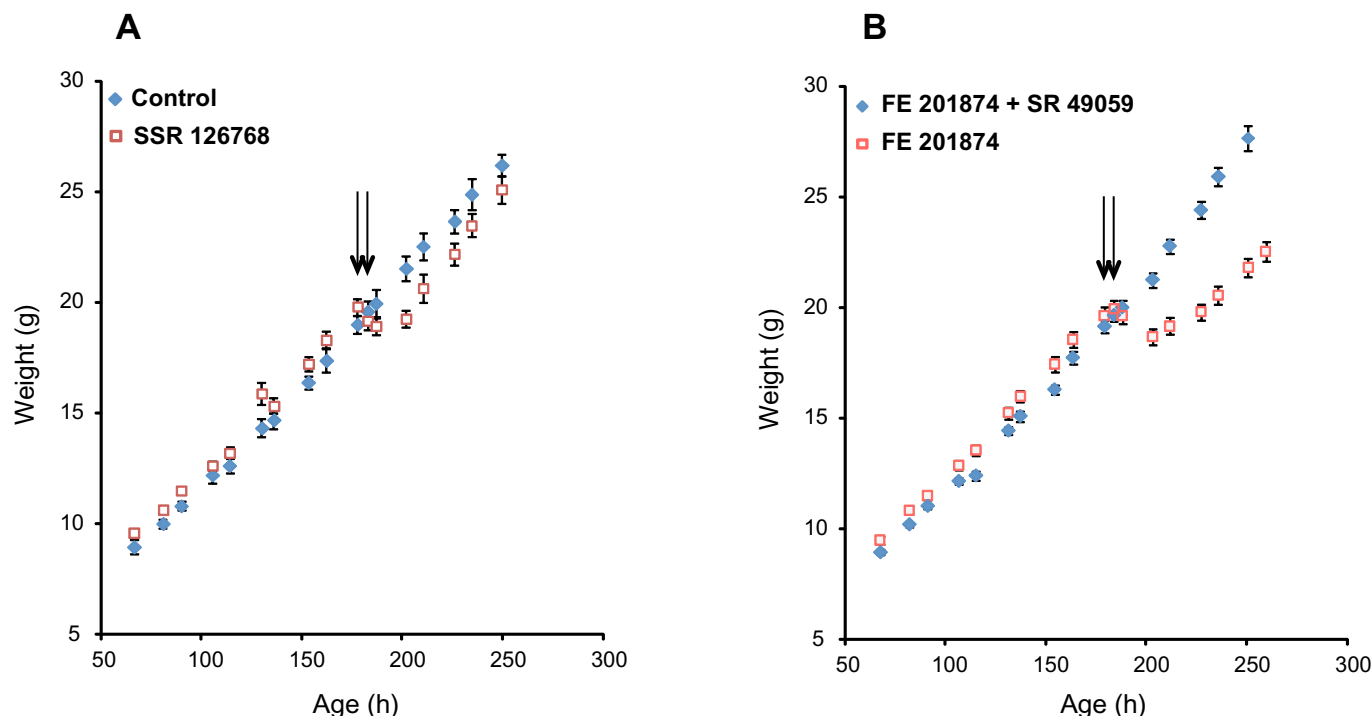## Figure 6

Mitogenic properties of FE 201874 on cell proliferation within the rat adrenal gland: comparison with selective AVP/OT agonists and antagonist. Rats (250 g) were implanted for 3 days with Alzet pump delivering increasing amounts of FE 201874, AVP or vehicle (control). Animals were injected with BrdU, fixed with paraformaldehyde and adrenal glands analysed for cell proliferation as described under Methods. (A) Illustrates the comparative effects of AVP (3.2 μg·day^{-1}) and FE 201874 (3.2 μg·day^{-1}) on BrdU-labelled nuclei within the rat adrenal cortex as compared to control rats. Scale bar represent 10 μm. (B) Illustrates cumulative dose-response curves for AVP, FE 201874, d[Leu^4,Lys^8]VP, [Thr^4,Gly^7]OT and dDAVP deduced from traces illustrated in (A) and from unshown results. Data are expressed as the number of BrdU-labelled nuclei counted per adrenal cortex; mean $\pm$ SEM of at least three experiments.

As illustrated in this paper, FE 201874 an analogue of F180, exhibits nanomolar affinity for the rV$_{1A}$ receptor in a variety of cellular contexts (WRK1 cell line or rat liver cells) and an excellent rV$_{1B}$/rV$_{1A}$ and rV$_2$/rV$_{1A}$ receptor selectivity and less evident selectivity towards the rOT receptor. A similar pharmacological profile was observed for human AVP/OT receptors. However, in contrast, FE 201874 was found to be non-selective and to have weak affinity for the mouse V$_{1A}$ receptor. Taken together, these observations

confirm the well-known pharmacological species differences already described for the AVP/OT receptor family (Busnelli et al., 2012). It is also interesting to note that only a few chemical modifications of the F180 molecule (replacement of neutral Hmp^1 with positively charged Cys and Dab(Abu)^8 with Orn) lead to rat V$_{1A}$ receptor selectivity. As the carboxy terminal tail of AVP is not directly in contact with the V$_{1A}$ receptor hormone binding pocket (Rodrigo et al., 2007), this probably implies that the human/rat selectivity largely

Figure 7

Influence of OT antagonist SSR126768A and FE 201874 on rat lactation. Lactation of rat dams (350 g) was evaluated by monitoring pups weight twice a day (24–32 pups from 3 to 4 dams per data point). Lactating dams were injected i.p. twice (0.5 ml; vertical arrows) with either NaCl (0.9%; Control), SSR126768A (3.3 mg·day^{-1}) or FE 201874 (0.13 mg·day^{-1}) on day 7. The V_{1A} agonist effect of FE 201874 was prevented by a pre-injection of SR49059 (6.6 mg·day^{-1}). Note that the OT antagonist induced a transient blockade of lactation resulting in a decreased pups growth. This effect was mimicked by FE 201 874 but this latter action was independent of the OT receptor since it was blocked by pre-injection of SR49059 (6.6 mg·day^{-1}), a specific V_{1A} receptor antagonist.

depends upon chemical interactions between the first cysteine of FE 201874 and the core of the rat V_{1A} receptor.

Functional studies performed on cell lines expressing rAVP receptors also indicate that FE 201874 is a full agonist of rV_{1A}, rV_{1B} and rV_2 receptors; the K_{act} are in accordance with the corresponding K_i determined by binding studies (see Tables 1 and 2) and corroborate the selectivity for the V_{1A} receptor. Experiments performed either *ex vivo* (rat aorta contraction) or *in vivo* (rat glomerulosa cell proliferation) also confirm these conclusions. FE 201874 is a full and selective V_{1A} agonist; whatever the biological model tested, K_{act} and maximal efficacy are similar, although slightly weaker, to that of the natural hormone AVP.

In contrast, in CHO cells expressing rOT receptors, FE 201874 only very weakly stimulated the basal production of InsPs and dose-dependently inhibited OT-stimulated InsP accumulation with a K_i related to its binding affinity for rOT receptor. Such OT antagonism properties have been previously described *in vitro* for F180 and its derived peptides on rat and human OT receptors (Andrès *et al.*, 2002; Laporte *et al.*, 2011). We found that, although FE 201874 mimics the action of an OT receptor antagonist *in vivo* on lactation, its mode of action actually involves V_{1A} receptors. We observed that the OT receptor antagonist SSR126768A (Serradeil-Le Gal *et al.*, 2004), delayed pups growth. A similar delay was obtained with FE 201874. Although a block of milk ejection is

probably induced by FE 201874 (as attested by the absence of milk in the pups' stomach, not shown), the action of this compound was completely blocked by pretreatment with the rat selective V_{1A} receptor antagonist SR49059. This result suggests that *in vivo*, this effect of FE 201874 is mediated mostly by stimulation of V_{1A} receptors and not by inhibition of OT receptors. Such an unexpected result is probably related to the previously described effect of vasoconstrictor drugs on mammary blood flow. As previously demonstrated in goats with adrenaline, reducing this blood flow very rapidly leads to a parallel decrease in milk production (Prosser *et al.*, 1996). As FE 201874 induces aorta ring contraction (this study) and increases arterial pressure (Wisniewski *et al.*, 2011), it may decrease mammary blood flow and, as a consequence, milk production and pup growth via stimulation of V_{1A} receptors. Several factors might have contributed to the failure to observe the involvement of OT receptor antagonism in the decrease in milk ejection in our dams (i) the dose of FE 201874 was limited by its vasoconstrictive effects on the animals; (ii) the effect of mammary blood flow reduction due to vasoconstriction might have overwhelmed the milk ejection reflex induced by endogenous oxytocin; and (iii) *in vivo*, FE 201874 might have lower affinity for the rat OT receptor as an antagonist than as an agonist at the rat V_{1A} receptor. Such a discrepancy has been observed for rat V_{1A} receptors expressed either in rat hepatocytes or in WRK1 cells (Cantau

et al., 1980; Guillon et al., 1986) and for F180, which behaves as a selective V_{1A} receptor agonist in vivo and a non-selective vasopressin agonist in vitro (Andrès et al., 2002). This result is further demonstrates that pharmacological results obtained in cellular models must be confirmed by in vivo experiments before being considered significant.

Other reasons with regard to the tissue and cellular context of the mammary gland could also underlie the results obtained. For example, FE 201874 availability at the mammary gland may be restricted by diffusion barriers or specific degradation. This hypothesis could not be tested since dams injected with a higher dose of FE 201874 showed signs of physical discomfort that precluded a normal relationship with the pups (not shown).

This new selective V_{1A} agonist may have potential as a treatment for the vasodilator-induced hypotension occurring in conditions such as septic shock (Maybauer and Maybauer, 2011) and it also appears to be the most suitable compound for discriminating between the behavioural effects of AVP and OT. Indeed, OT, V_{1A} and V_{1B} receptors have been shown to be present in many areas of the rodent brain and numerous behavioural studies have demonstrated opposite roles of OT and AVP in sociality and bonding, as well as in anxiety, fear and response to stress (Caldwell et al., 2008; Choleris et al., 2009; Stoop, 2012). Up to now, the lack of a selective rat V_{1A} receptor agonist has hampered these studies, due to the presence of V_2 receptor-mediated side effects such as vasodilatation and coagulation factor release (Kaufmann and Vischer, 2003) or to the poor selectivity of the AVP/OT drugs used. For example, the use of the Manning compound to demonstrate a V_{1A} receptor-mediated effect of AVP on rat memory is not satisfactory since the peptide is a mixed V_{1A}/OT receptor antagonist. Similarly, as SR 149415 is a mixed V_{1B}/OT antagonist (Griffante et al., 2005), it cannot be used to demonstrate that stress involves activation of V_{1B} receptors. Also, the nature of the AVP receptors mediating LTP in the rat CA2 hippocampus area (Chafai et al., 2012) remains to be evaluated using the selective V_{1B} (d[Leu^4,Lys^8]VP) and V_{1A} (FE 201874) agonists now available, since many pharmacological and immunological data strongly suggest the presence of both receptors in this area (Vaccari et al., 1998; Hernando et al., 2001; Young et al., 2006).

The development of specific peptidic or non-peptidic drugs to decipher which AVP/OT receptor isoform is responsible for a given physiological function has constituted an active research field for more than two decades (Manning et al., 2012). Thus, [Thr^4,Gly^7]OT, d[Leu^4,Lys^8]VP, FE 202158 (selepressin), and to a lesser extent dDAVP are respectively considered as selective OT, V_{1B}, V_{1A} and V_2 receptor agonists (Guillon et al., 2004; Pena et al., 2007; Laporte et al., 2011). Similarly, compounds like SR49059, SR121463A and SSR126415A have been used to selectively antagonize the functional consequences of V_{1A}, V_2 and OT receptor activation (for review see Chini et al., 2008). In addition to its usefulness for animal research, the development of selective compounds may lead to valuable tools with potential therapeutic interest. Indeed desmopressin, a selective V_2 receptor agonist, is widely used for the treatment of enuresis and central diabetes insipidus.

In summary, these data demonstrate that FE 201874 is a full rV_{1A} receptor agonist in the biological models tested.

Moreover, this peptide discriminates very well between rV_{1A} and rV_2 or rV_{1B} receptors. Its relatively weak V_{1A}/OT selectivity, as revealed by binding experiments, is counterbalanced by its OT antagonistic properties. Indeed, as FE 201874 does not stimulate the OT receptor, its binding to this receptor isoform will have no functional consequences and will, in fact, improve its selectivity for V_{1A} receptors in functional studies. In view of the selectivity indices of FE 201874, in experiments on cell cultures or tissues slices concentrations exceeding 10nM or 100nM, respectively, should not be used if a selective action on the rV_{1A} receptor is required. A similar specificity will be obtained in in vivo experiments with doses under 12 µg·kg^{-1}·day^{-1}.

In conclusion, the present data, obtained from a combination of binding and physiological experiments, demonstrate that FE 201874 is a specific V_{1A} receptor agonist in the rat.

Acknowledgements

Conflict of interest

Authors declare that they have not any conflict of interest.

References

Abou-Samra AB, Harwood JP, Manganiello VC, Catt KJ, Aguilera G (1987). Phorbol 12-myristate 13-acetate and vasopressin potentiate the effect of corticotropin-releasing factor on cyclic AMP production in rat anterior pituitary cells. Mechanisms of action. J Biol Chem 262: 1129–1136.

Alexander SPH, Mathie A, Peters JA (2011). Guide to receptors and channels (GRAC), 5th edition. Br J Pharmacol 164 (Suppl. 1): S1–S324.

Alonso G, Galibert E, Boulay V, Guillou A, Jean A, Compan V et al. (2009). Sustained elevated levels of circulating vasopressin selectively stimulate the proliferation of kidney tubular cells via the activation of V2 receptors. Endocrinology 150: 239–250.

Andrès M, Trueba M, Guillon G (2002). Pharmacological characterization of F-180: a selective human V(1a) vasopressin receptor agonist of high affinity. Br J Pharmacol 135: 1828–1836.

Aurell CJ, Bengtsson B, Ekholm K, Kasprzykowska R, Nilsson A, Persson R et al. (1991). Development of vasopressor specific vasotocin analogs with prolonged effects. In: Giralt E, Andreu D (eds). Peptides 1990, Proceedings of the 21st European Peptide Symposium. Springer: Barcelona, pp. 671–673.

Barberis C, Audigier S, Durroux T, Elands J, Schmidt A, Jard S (1992). Pharmacology of oxytocin and vasopressin receptors in the central and peripheral nervous system. Ann N Y Acad Sci 652: 39–45.

Busnelli M, Sauliere A, Manning M, Bouvier M, Gales C, Chini B (2012). Functional selective oxytocin-derived agonists discriminate between individual G protein family subtypes. J Biol Chem 287: 3617–3629.

Caldwell HK, Lee HJ, Macbeth AH, Young WS, 3rd (2008). Vasopressin: behavioral roles of an 'original' neuropeptide. Prog Neurobiol 84: 1–24.

Cantau B, Keppens S, De Wulf H, Jard S (1980). (3H)-vasopressin binding to isolated rat hepatocytes and liver membranes: regulation by GTP and relation to glycogen phosphorylase activation. J Recept Res 1: 137–168.

Chafai M, Corbani M, Guillon G, Desarménien MG (2012). Vasopressin inhibits LTP in the CA2 mouse hippocampal area. PLoS ONE 7: e49708.

Cheng Y, Prusoff WH (1973). Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem Pharmacol 22: 3099–3108.

Chini B, Manning M, Guillon G (2008). Affinity and efficacy of selective agonists and antagonists for vasopressin and oxytocin receptors: an 'easy guide' to receptor pharmacology. Prog Brain Res 170: 513–517.

Choleris E, Clipperton-Allen AE, Phan A, Kavaliers M (2009). Neuroendocrinology of social information processing in rats and mice. Front Neuroendocrinol 30: 442–459.

Cordaillat M, Fort A, Virsolvy A, Elghozi J-L, Richard S, Jover B (2007). Nitric oxide pathway counteracts enhanced contraction to membrane depolarization in aortic rings of rats on high-sodium diet. Am J Physiol Regul Integr Comp Physiol 292: R1557–R1562.

De Wied D (1971). Long term effect of vasopressin on the maintenance of a conditioned avoidance response in rats. Nature 232: 58–60.

Deblon N, Veyrat-Durebex C, Bourgoin L, Caillon A, Bussier A-L, Petrosino S et al. (2011). Mechanisms of the anti-obesity effects of oxytocin in diet-induced obese rats. PLoS ONE 6: e25565.

Derick S, Cheng LL, Voirol MJ, Stoev S, Giacomini M, Wo NC et al. (2002). [1-deamino-4-cyclohexylalanine] arginine vasopressin: a potent and specific agonist for vasopressin V1b receptors. Endocrinology 143: 4655–4664.

Gillies GE, Linton EA, Lowry PJ (1982). Corticotropin releasing activity of the new CRF is potentiated several times by vasopressin. Nature 299: 355–357.

Grazzini E, Boccara G, Joubert D, Trueba M, Durroux T, Guillon G et al. (1998). Vasopressin regulates adrenal functions by acting through different vasopressin receptor subtypes. Adv Exp Med Biol 449: 325–334.

Griffante C, Green A, Curcuruto O, Haslam CP, Dickinson BA, Arban R (2005). Selectivity of d[Cha4]AVP and SSR149415 at human vasopressin and oxytocin receptors: evidence that SSR149415 is a mixed vasopressin V1b/oxytocin receptor antagonist. Br J Pharmacol 146: 744–751.

Guillon G, Mouillac B, Balestre MN (1986). Activation of polyphosphoinositide phospholipase C by fluoride in WRK1 cell membranes. FEBS Lett 204: 183–188.

Guillon G, Derick S, Pena A, Cheng LL, Stoev S, Seyer R et al. (2004). The discovery of novel vasopressin V1b receptor ligands for pharmacological, functional and structural investigations. J Neuroendocrinol 16: 356–361.

Guillon G, Pena A, Murat B, Derick S, Trueba M, Ventura MA et al. (2006). Position 4 analogues of [deamino-Cys(1)] arginine vasopressin exhibit striking species differences for human and rat V(2)/V(1b) receptor selectivity. J Pept Sci 12: 190–198.

Gutkowska J, Jankowski M (2012). Oxytocin revisited: its role in cardiovascular regulation. J Neuroendocrinol 24: 599–608.

Hernando F, Schoots O, Lolait SJ, Burbach JP (2001). Immunohistochemical localization of the vasopressin V1b receptor in the rat brain and pituitary gland: anatomical support for its involvement in the central effects of vasopressin. Endocrinology 142: 1659–1668.

Jard S (1998). Vasopressin receptors. A historical survey. Adv Exp Med Biol 449: 1–13.

Kassis S, Henneberry RC, Fishman PH (1984). Induction of catecholamine-responsive adenylate cyclase in HeLa cells by sodium butyrate. Evidence for a more efficient stimulatory regulatory component. J Biol Chem 259: 4910–4916.

Kaufmann JE, Vischer UM (2003). Cellular mechanisms of the hemostatic effects of desmopressin (DDAVP). J Thromb Haemost 1: 682–689.

Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2011). Animal research: reporting in vivo experiments–the ARRIVE guidelines. J Cereb Blood Flow Metab 31: 991–993.

Kirk CJ, Guillon G, Balestre MN, Jard S (1986). Stimulation, by vasopressin and other agonists, of inositol-lipid breakdown and inositol phosphate accumulation in WRK 1 cells. Biochem J 240: 197–204.

Koshimizu T-A, Nakamura K, Egashira N, Hiroyama M, Nonoguchi H, Tanoue A (2012). Vasopressin v1a and v1b receptors: from molecules to physiological systems. Physiol Rev 92: 1813–1864.

Laporte R, Kohan A, Heitzmann J, Wisniewska H, Toy J, La E et al. (2011). Pharmacological characterization of FE 202158, a novel, potent, selective, and short-acting peptidic vasopressin V1a receptor full agonist for the treatment of vasodilatory hypotension. J Pharmacol Exp Ther 337: 786–796.

Lee B, Yang C, Chen TH, al-Azawi N, Hsu WH (1995). Effect of AVP and oxytocin on insulin release: involvement of V1b receptors. Am J Physiol 269: E1095–E1100.

McGrath JC, Drummond GB, McLachlan EM, Kilkenny C, Wainwright CL (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Br J Pharmacol 160: 1573–1576.

Manning M, Misicka A, Olma A, Bankowski K, Stoev S, Chini B et al. (2012). Oxytocin and vasopressin agonists and antagonists as research tools and potential therapeutics. J Neuroendocrinol 24: 609–628.

Maybauer MO, Maybauer DM (2011). Vasopressin analogues and V1a receptor agonists in septic shock. Inflamm Res 60: 425–427.

Meyer-Lindenberg A, Domes G, Kirsch P, Heinrichs M (2011). Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. Nat Rev Neurosci 12: 524–538.

Murat B, Devost D, Andrés M, Mion J, Boulay V, Corbani M et al. (2012). V1b and CRHR1 receptor heterodimerization mediates synergistic biological actions of vasopressin and CRH. Mol Endocrinol 26: 502–520.

Pena A, Murat B, Trueba M, Ventura MA, Bertrand G, Cheng LL et al. (2007). Pharmacological and physiological characterization of d[Leu(4), Lys(8)] vasopressin, the first V1b-Selective agonist for rat vasopressin/oxytocin receptors. Endocrinology 148: 4136–4146.

Prosser CG, Davis SR, Farr VC, Lacasse P (1996). Regulation of blood flow in the mammary microvasculature. J Dairy Sci 79: 1184–1197.

Rodrigo J, Pena A, Murat B, Trueba M, Durroux T, Guillon G et al. (2007). Mapping the binding site of arginine vasopressin to V1a and V1b vasopressin receptors. Mol Endocrinol 21: 512–523.

Ryckwaert F, Virsolvy A, Fort A, Murat B, Richard S, Guillon G et al. (2009). Terlipressin, a provasopressin drug exhibits direct vasoconstrictor properties: consequences on heart perfusion and performance. Crit Care Med 37: 876–881.

Serradeil-Le Gal C, Wagnon J, Garcia C, Lacour C, Guiraudou P, Christophe B et al. (1993). Biochemical and pharmacological properties of SR 49059, a new, potent, nonpeptide antagonist of rat and human vasopressin V1a receptors. J Clin Invest 92: 224–231.

Serradeil-Le Gal C, Valette G, Foulon L, Germain G, Advenier C, Naline E et al. (2004). SSR126768A (4-chloro-3-[(3R)-(+)-5-chloro-1-(2,4-dimethoxybenzyl)-3-methyl-2-oxo-2,3-dihydro-1H-indol-3-yl]-N-ethyl-N-(3-pyridylmethyl)-benzamide, hydrochloride): a new selective and orally active oxytocin receptor antagonist for the prevention of preterm labor. J Pharmacol Exp Ther 309: 414–424.

Stoop R (2012). Neuromodulation by oxytocin and vasopressin. Neuron 76: 142–159.

Thibonnier M (2004). Genetics of vasopressin receptors. Curr Hypertens Rep 6: 21–26.

Vaccari C, Lolait SJ, Ostrowski NL (1998). Comparative distribution of vasopressin V1b and oxytocin receptor messenger ribonucleic acids in brain. Endocrinology 139: 5015–5033.

Wisniewski K, Galyean R, Tariga H, Alagarsamy S, Croston G, Heitzmann J et al. (2011). New, potent, selective, and short-acting peptidic V1a receptor agonists. J Med Chem 54: 4388–4398.

Wooten DL, Simms J, Massoura AJ, Trim JE, Wheatley M (2011). Agonist-specific requirement for a glutamate in transmembrane helix 1 of the oxytocin receptor. Mol Cell Endoc 333: 20–27.

Yibchok-Anun S, Cheng H, Heine PA, Hsu WH (1999). Characterization of receptors mediating AVP- and OT-induced glucagon release from the rat pancreas. Am J Physiol 277: E56–E62.

Young WS, Li J, Wersinger SR, Palkovits M (2006). The vasopressin 1b receptor is prominent in the hippocampal area CA2 where it is unaffected by restraint stress or adrenalectomy. Neuroscience 143: 1031–1039.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Chemicals.