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Pharmacological Characterization of FE 202158, a Novel, Potent, Selective, and Short-Acting Peptidic Vasopressin V_{1a} Receptor Full Agonist for the Treatment of Vasodilatory Hypotension

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ABSTRACT

FE 202158, ([Phe^2,Ile^3,Hgn^4,Orn(iPr)^8]vasopressin, where Hgn is homoglutamine and iPr is isopropyl), a peptidic analog of the vasoconstrictor hormone [Arg^8]vasopressin (AVP), was designed to be a potent, selective, and short-acting vasopressin type 1a receptor ($V_{1a}R$) agonist. In functional reporter gene assays, FE 202158 was a potent and selective human $V_{1a}R$ agonist [EC_{50} = 2.4 nM; selectivity ratio of 1:142:1107:440 versus human vasopressin type 1b receptor, vasopressin type 2 receptor (V_2R), and oxytocin receptor, respectively] contrasting with AVP's lack of selectivity, especially versus the V_2R (selectivity ratio of 1:18:0.2:92; human $V_{1a}R$ EC_{50} = 0.24 nM). This activity and selectivity profile was confirmed in radioligand binding assays. FE 202158 was a potent vasoconstrictor in the isolated rat common iliac artery ex vivo (EC_{50} = 3.6 nM versus 0.8 nM for AVP) and reduced rat ear skin blood flow after intravenous infusion in vivo (ED_{50} = 4.0 versus 3.4 pmol/kg/min for AVP). The duration of its vasopressor effect by intravenous bolus in rats was as short as AVP at submaximally effective doses. FE 202158 had no V_2R-mediated antidiuretic activity in rats by intravenous infusion at its ED_{50} for reduction of ear skin blood flow, in contrast with the pronounced antidiuretic effect of AVP. Thus, FE 202158 seems suitable for treatment of conditions where $V_{1a}R$ activity is desirable but V_2R activity is potentially deleterious, such as vasodilatory hypotension in septic shock. In addition to the desirable selectivity profile, its short-acting nature should allow dose titration with rapid onset and offset of action to optimize vasoconstriction efficacy and safety.

Introduction

Sepsis is at its essence an excessive systemic inflammatory response to numerous infectious and noninfectious causes (e.g., trauma, burns, pancreatitis, and cardiopulmonary bypass) that may progress to multiple organ failure and death. It is the 10th-leading cause of death in the United States (2008), and its incidence has dramatically increased over the past decade and is expected to continue growing in large part because of an increased number of multiresistant bacteria, more immunosuppressed patients, and the aging population (Hodgin and Moss, 2008). In addition to antibiotics, standard of care includes fluid resuscitation and/or vasopressor drugs to treat the severe hypotension that develops as sepsis progresses to septic shock as a consequence of sepsis-induced systemic vasodilation and vascular/capillary leak (Annane et al., 2005; Russell, 2007).

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ABBREVIATIONS: AVP, [Arg^8]vasopressin; CI, confidence interval; CCRC, cumulative concentration-response curves; C_{H2O}, free water clearance; CHO, Chinese hamster ovary; DAP, diastolic arterial pressure; E_{max}, maximal possible response produced by an agonist or an antagonist; EWC, effective water clearance; Hgn, homoglutamine; Hmp, 2-hydroxy-3-mercaptopropionic acid; F-180, [Hmp^1,Phe^2,Hgn^4,Dbu(Abu)^8]vasotocin; iPr, isopropyl; FE 202158, [Phe^2,Ile^3,Hgn^4,Orn(iPr)^8]vasopressin; HEK, human embryonic kidney; Luc, luciferase; NFAT, nuclear factor of activated T cells; n_H, Hill coefficient; OT, oxytocin; OTR, OT receptor; pA_2, negative logarithm of the concentration of antagonist needed to shift the CCRC by a factor of 2; PSS, physiological salt solution; U_{osm}, urine osmolality; $V_{1a}R$, vasopressin type 1a receptor; $V_{1b}R$, vasopressin type 1b receptor; V_2R, vasopressin type 2 receptor; U.S.P., United States Pharmacopeia; U-50488, 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1*R*,2*R*)-2-pyrrolidin-1-ylcyclohexyl]acetamide; Pmp, β,β-cyclopentamethylene-β-mercaptopropionyl.

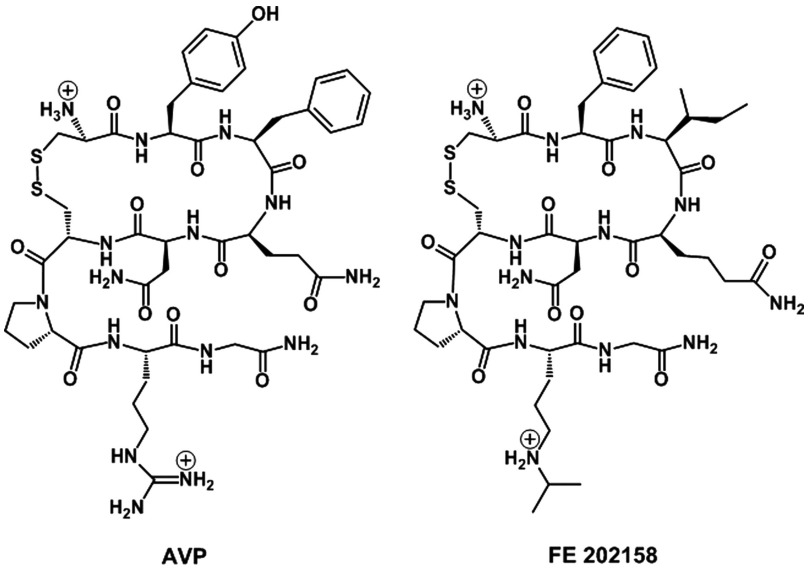

Fig. 1. Chemical structures of AVP (left) and FE 202158 (right).

Clinical studies have documented that a deficiency in the plasma concentration of the vasoconstrictor neurohypophyseal hormone [Arg^8]vasopressin (AVP) may develop in septic shock (Landry et al., 1997a; Jochberger et al., 2009). This led to the hypothesis by Landry and collaborators, who made the initial observation (Landry et al., 1997a) that AVP administration in septic shock may be considered as replacement hormone therapy (Oliver and Landry, 2007). Numerous small pilot studies, starting with one from Landry et al. (1997b), indeed have showed that "add-back" vasopressin therapy (i.e., continuous low-dose intravenous infusion of 0.01–0.04 U/min) may reverse hypotension in norepinephrine-resistant septic shock (Holmes and Walley, 2008). The first large-scale multicenter study, the Vasopressin in Septic Shock Trial, has suggested that "add-back" vasopressin therapy (0.03 U/min) may reduce mortality in less severe septic shock (Russell et al., 2008).

AVP is the endogenous ligand for the three known subtypes of vasopressin receptor, $V_{1a}R$, $V_{1b}R$ (V_3R), and V_2R, with $V_{1a}R$ and V_2R being the principal subtypes mediating its physiological functions (Barberis et al., 1998); it can also act on the oxytocin receptor (OTR) at high concentrations. Whereas the $V_{1a}R$ mediates vasoconstriction (Holmes et al., 2003), the V_2R mediates effects that would be deleterious in sepsis, such as water retention through antidiuresis (Knepper, 1997), vasodilation (Kaufmann and Vischer, 2003), and coagulation factor release (Kaufmann and Vischer, 2003). This led to our hypothesis that a selective $V_{1a}R$ agonist would be superior to the mixed $V_{1a}R/V_2R$ agonist AVP in treating vasodilatory hypotension occurring in conditions of vasodilatory hypotension such as septic shock. We further hypothesized that this agonist would need to remain as short-acting a vasopressor as AVP to permit dose titration with rapid onset and offset of action to optimize vasoconstriction efficacy and safety. Thus, a long-acting selective $V_{1a}R$ agonist such as F-180 [Hmp^1,Phe^2,Hgn^4,Dbu(Abu)^8]vasotocin, where Hmp is 2-hydroxy-3-mercaptopropionic acid and Hgn is homoglutamine] (Aurell et al., 1991; Bernadich et al., 1998; Andrés et al., 2002) would not be suitable.

Here, we describe the primary in vitro, ex vivo, and in vivo pharmacological characterization of the novel, potent, selective, and short-acting peptidic vasopressin $V_{1a}R$ agonist, FE 202158 ([Phe^2,Ile^3,Hgn^4,Orn(iPr)^8]vasopressin, where iPr is isopropyl) (Fig. 1) (Wisniewski et al., 2009). We report in vitro functional and binding activity, data from ex vivo and in vivo rat models of vasoconstriction, and pharmacokinetic characterization in rats. Some of these results have been presented previously (Laporte et al., 2008a).

Materials and Methods

Binding Affinity and Selectivity at the Recombinant Human Vasopressin and Oxytocin Receptors

FE 202158 in vitro binding activity was characterized compared with AVP at the recombinant human $V_{1a}R$, $V_{1b}R$, V_2R, and OTR. Membranes from human embryonic kidney (HEK) 293 cells transfected with cDNAs encoding for the human $V_{1a}R$, $V_{1b}R$, V_2R, or OTR were isolated. The membrane proteins were coupled to poly-(vinyl toluene)-wheat germ agglutinin scintillation proximity assay beads (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK) overnight at 4°C. After wash, the membrane-bead suspension was incubated with or without competitor compounds and radioligand [[^{3}H]AVP (0.5–3 nM) or ^{125}I-ornithine vasotocin (0.075–0.20 nM)] in a total volume of 100 μl for 30 min at room temperature. The signal was detected using a Wallac Microbeta Trilux counter (PerkinElmer Life and Analytical Sciences, Waltham, MA). To measure the binding affinity of FE 202158 and AVP, binding displacement experiments were performed in the presence of increasing concentrations of the respective test ligand (0.01 nM–10 μM). Total binding was defined as the signal detected in the absence of test ligand. Nonspecific binding was determined in the presence of excess unlabeled reference ligand (10 μM AVP for $V_{1a}R$, $V_{1b}R$, and V_2R; 10 μM OT for OTR). Specific binding was obtained by subtraction of nonspecific binding from the signal detected. For each receptor type assay, radioligand displacement by the test ligand was expressed as percentage of maximal radioligand displacement by the unlabeled reference ligand.

Functional Agonist Activity at the Recombinant Vasopressin and Oxytocin Receptors

FE 202158 in vitro functional agonist activity was characterized compared with AVP at the recombinant human $V_{1a}R$, $V_{1b}R$, V_2R, and OTR. It was also assessed at the recombinant mouse, rat, dog, sheep,

and pig $V_{1a}R$ and V_2R to confirm that FE 202158 selectivity was preserved/retained across these animal species. Specifically, this activity was evaluated in cell-based reporter gene assays in HEK-293 or Chinese hamster ovary (CHO) K1 (CHO-K1) cells transiently transfected by use of Lipofectamine reagent (Invitrogen, Carlsbad, CA) with the plasmid DNAs encoding either the $V_{1a}R$ or V_2R gene from all the animal species listed above together with a luciferase reporter gene under control of transcriptional regulatory elements responsive to receptor activation, with the exception of the mouse $V_{1a}R$ assay that used a stable cell line (Flp-In-293 host cells; Invitrogen; Table 1). The assays for human $V_{1b}R$ and OTR were conducted in a similar way except using stable cell lines (human $V_{1b}R$ in Flp-In-293 host cells (Invitrogen) and human OTR in CHO-K1 host cells). Agonist activity was determined 24 to 48 h after transfection by measuring expression of the reporter gene using the Luclite Reporter Gene Assay System, (PerkinElmer Life and Analytical Sciences) after 5 h of agonist exposure at a concentration range of 0.1 pM to 10 μM.

Animals

Wistar rats (200–328 g) were obtained from Harlan (Indianapolis, IN). Upon delivery, rats were housed two per microisolator cage at the Ferring Research Institute animal care facility under controlled environmental conditions (12-h light/dark cycle, lights on at 7 AM; 22–25°C) in a small animal housing cabinet (Scantainer Classic; Scanbur A/S, Karlslunde, Denmark) or a positive-pressure clean room (bioBubble, Fort Collins, CO) with free access to standard rodent chow (5001 rodent diet; PMI Nutrition International, Richmond, IN or 18% protein rodent diet; Harlan Teklad, Madison, WI) and acidified tap water (pH 2.5–3.0). Housing conditions were in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Research Council. The rats were allowed to acclimate for at least 2 days before study use. The in vivo portion of all study protocols was approved by our Institutional Animal Care and Use Committee.

Contractile Activity in the Isolated Rat Common Iliac Artery

Vasoconstriction was assessed compared with AVP ex vivo in the isolated rat common iliac artery, a blood vessel very responsive to the contractile effect of $V_{1a}R$ agonists (Ramírez et al., 2001). In this assay, we also assessed the sensitivity of FE 202158 vasoconstrictive effect to a selective $V_{1a}R$ antagonist to demonstrate the receptor subtype-specific mediation of the effect and the sensitivity to two pharmacological classes of antihypertensive drugs (the L-type voltage-gated calcium channel blocker nifedipine and the soluble guanylate cyclase activator sodium nitroprusside) commonly available in critical-care medical settings where FE 202158 is intended to be used to demonstrate that such drugs, as functional antagonists, would be efficacious "antidotes" in case of accidental overdosing of the compound.

In brief, male rats were euthanized by CO_2 inhalation, and common iliac arteries were immediately excised and placed into a modified Krebs physiological salt solution (PSS) of the following composition: 120 mM NaCl, 4.6 mM KCl, 1.5 mM $NaH_2PO_4{\cdot}1H_2O$, 0.7 mM Na_2HPO_4, 11.5 mM D-glucose, 25 mM $NaHCO_3$, 2.4 mM $CaCl_2$, 1.2 mM $MgCl_2$, pH 7.35 to 7.45. After cleaning of adherent tissues under a dissecting microscope in carbogen-aerated PSS maintained at 37°C, each common iliac artery was cut to a ring of a little over 2 mm in length. The rings were mounted in 10-ml tissue baths (DMT Multi Myograph System 610M; Danish Myo Technology, Aarhus, Denmark) containing carbogen-aerated PSS maintained at 37°C with isometric force continuously acquired with Notocord-hem Evolution (Notocord Systems SAS, Croissy sur Seine, France), and progressively stretched to their optimal length (1.48 times their slack length) over 30 to 60 min. The rings' contractile activity was then stabilized with three consecutive exposures to a maximally effective potassium-induced depolarization (80 mM K^+ PSS obtained by appropriate isotonic substitution of NaCl by KCl). Endothelium function was tested with acetylcholine (10 μM)-induced relaxation of the vascular rings during the steady-state contraction phase of the third stimulation with the 80 mM K^+ PSS. Cumulative concentration-response curves (CCRC) by half-log concentration increase to AVP (0.03–100 nM) or FE 202158 (0.1–10,000 nM) were obtained with only a single CCRC for a given agonist built in a given vascular ring.

For the pA_2 determination for the selective $V_{1a}R$ antagonist [Pmp^1,$Tyr(Me)^2$,Arg^8]vasopressin (Ohlstein and Berkowitz, 1986), where pA_2 is the negative logarithm of the concentration of antagonist needed to shift the CCRC by a factor of 2, one ring from a given rat was exposed to a concentration of the antagonist (0.1, 1, or 10 nM) for 15 min, while the ring from the opposite common iliac artery from the same rat was used as antagonist-free control and received PSS instead (preliminary study showed that the potency and efficacy of AVP were similar between left and right common iliac arteries in a given rat). A CCRC for FE 202158 was then built in each ring and the contractile response of the antagonist-treated ring was expressed as a percentage of the maximal response of the opposite control ring.

For the IC_{50} determination for nifedipine and sodium nitroprusside, a reference contraction was induced with 5.6 nM FE 202158 instead of building a CCRC. Contralateral vascular rings from a given rat were then treated differently; one ring was exposed to 0.1 nM nifedipine for at least 5 min or 1 nM sodium nitroprusside for at least 15 min while the contralateral ring was exposed to PSS and used as time controls for the stability of the repeated contractile responses to 5.6 nM FE 202158. All rings were then re-exposed to 5.6 nM FE 202158 in the presence of the functional antagonist or PSS. This cycle was repeated with 1, 10, 100, and 1000 nM nifedipine or 10, 100, 1000, and 10,000 nM sodium nitroprusside. For each vascular ring, contractile responses were expressed as a percentage of the initial nifedipine/sodium nitroprusside-free FE 202158-induced response corrected for time-dependent changes of the FE 202158-induced contraction in the contralateral control ring and converted into percentage of inhibition of this initial FE 202158 response.

Pharmacokinetics in Conscious Rats

Single-dose pharmacokinetics of FE 202158 was investigated after intravenous bolus administration to male rats that had chronic jug-

TABLE 1
Recombinant human vasopressin and oxytocin receptor cell-based luciferase reporter gene assays

Transfection		Host Cells	Receptor Expression	Internal Reference
Receptor	Reporter			
None	NFAT_Luc	HEK-flpin-mV_{1a}R	Stable	AVP
Rat, sheep, and pig $V_{1a}R$	NFAT_Luc	HEK-293	Transient	AVP
Dog $V_{1a}R$	NFAT_Luc	CHO-K1	Transient	AVP
Human $V_{1a}R$	NFAT_Luc	HEK-293	Transient	AVP
None	NFAT_Luc	HEK-flpin-hV_{1b}	Stable	AVP
Mouse, rat, dog, sheep, and pig V_2R	CRE_Luc	HEK-293	Transient	dDAVP
HumanV_2R	CRE_Luc	HEK-293	Transient	dDAVP
None	NFAT_Luc	CHO-hOTR	Stable	Carbetocin

CRE, cAMP response element; dDAVP, desmopressin acetate.

ular vein and carotid artery catheters inserted surgically. Each rat was given a single dose of 3.0 μg/kg (2.9 nmol/kg) of the test article as a 3.0 μg/ml solution in sterile saline through the jugular vein. Blood samples (250 μl) were collected from the carotid artery at 1, 3, 5, 8, 12, 20, 30, 40, and 50 min after administration. Blood was replaced with an equal volume of sterile heparinized saline (20 U/ml). The samples were centrifuged, and plasma portions were separated. All samples were immediately frozen on dry ice and stored at −80°C until analysis. The concentrations of FE 202158 were determined using liquid chromatography/tandem mass spectrometry. The dynamic range of the assays was generally between 0.05 and 200 ng/ml. In brief, aliquots of rat plasma containing internal standard were precipitated with an equal volume of 0.1% trifluoroacetic acid in acetonitrile and centrifuged. The resulting supernatant solution was filtered through a 0.22-μm Amicon Ultrafree-MC filter (Millipore Corporation, Billerica, MA). Samples were injected into a Vydac C18 low trifluoroacetic acid 150 × 2.1 mm column (Grace, Deerfield, IL) coupled to an Agilent 1100 series LC (Agilent Technologies, Santa Clara, CA). The analytes were eluted by a mobile-phase gradient containing 0.01% trifluoroacetic acid and acetonitrile and detected using a Finnigan TSQ Quantum Ultra (Thermo Fisher Scientific, Waltham, MA) triple quadrupole mass spectrometer in the positive electrospray ionization mode. Analyte concentrations were calculated by linear regression analysis using the peak area ratio of analyte to the internal standard.

Vasoconstrictive Activity in Anesthetized Rats

To evaluate the translation of the ex vivo vasoconstrictive effect of FE 202158 (see *Contractile Activity in the Isolated Rat Common Iliac Artery*) to the in vivo setting, we assessed the effect of incremental intravenous infusion rates of the compound on ear blood flow in rats in comparison with AVP. The use of such a continuous intravenous infusion was allowing the establishment of incremental steady-state plasma concentration levels of FE 202158 and avoiding the complications of pharmacokinetic considerations in the assessment of this in vivo pharmacodynamic effect. Specifically, male rats were anesthetized with isoflurane (2.0–2.5% in oxygen), and anesthesia was maintained with thiobutabarbital sodium (150 mg/kg intraperitoneally, redosed at 50 mg/kg if needed). The rats were mechanically ventilated with 36% oxygen compressed air (HSE-HA Multiple-Channel Ventilator; Harvard Apparatus, Inc., Holliston, MA), and their body temperature was maintained at 37 to 38°C using a water circulating heating pad (MUL-T-PAD; Gaymar Industries, Orchard Park, NY). A carotid artery was catheterized to monitor arterial blood pressure (disposable pressure transducer sets DTX Plus TNF-R; BD Biosciences, Franklin Lakes, NJ) and the opposite jugular vein was catheterized for vehicle and drug administration. A laser doppler probe (Transonic Flow Probe Type N-and BLF21 Series Laser Doppler Monitor; Transonic Systems, Inc., Ithaca, NY) was placed on the ventral face of the ear pinna to monitor skin blood flow noninvasively. Arterial pressure and blood flow data were continuously acquired with Notocord-hem (Notocord Systems SAS). After stable hemodynamic recordings were established (~15–30 min), animals were infused with 1) heparinized vehicle solution (20 unit/ml heparin in 5% (w/v) dextrose and 0.45% (w/v) NaCl) for ~5 min to assess any nonspecific hemodynamic effects, followed by 2) an infusion of AVP (10 pmol/kg/min) lasting until skin blood flow was reduced to a stable level (~20–30 min) to assess vasoconstrictive and vasopressive integrity of the animal preparation, 3) vehicle infusion until a stable baseline ear blood flow was restored (~30 min), 4) a second infusion of AVP also at 10 pmol/kg/min to induce an internal reference response, and 5) a final infusion of vehicle to restore baseline blood flow and use as baseline for the dose-response curve to either FE 202158 (1–300 pmol/kg/min) or AVP (0.3–300 pmol/kg/min) that was then built incrementally (~30–45 min/dose). All infusion volume rates were maintained at 16.7 μl/kg/min (~5 μl/min; Genie syringe pumps; Kent Scientific, Torrington, CT). The change of ear skin blood flow in response to agonist infusion was expressed as a percentage of reduction from baseline level. Only one single dose-response curve was built in a given rat. The total duration of an experiment from induction of anesthesia was ~8 h.

Vasopressor Activity in Anesthetized Rats

We determined the duration of the vasopressive effect (i.e., the increase in arterial pressure) induced by intravenous bolus administration of FE 202158, a translation of its primary vasoconstrictive effect, compared with the duration of action of equieffective doses of the short-acting AVP and the long-acting and selective $V_{1a}R$ agonist F-180 (Aurell et al., 1991; Andrés et al., 2002). Specifically, male rats were anesthetized, mechanically ventilated, catheterized and placed on a 37°C heating pad as described above (see *Vasoconstrictive Activity in Anesthetized Rats*). Arterial pressure data were continuously acquired with Notocord-hem (Notocord Systems SAS). Before agonist administration, rats were pretreated intravenously with the α-adrenergic receptor antagonist Dibenamine (1.8 mg/rat administered divided into six boluses spread over 30 min) to stabilize arterial blood pressure and enhance vasopressor responsiveness to $V_{1a}R$ agonists (Dekanski, 1952). After stable hemodynamic recordings were established (~45 min), animals were administered intravenous 0.5 ml/kg boluses of 1) vehicle solution (20 units/ml heparin in 2.5% (w/v) dextrose and 0.45% (w/v) NaCl) to assess any nonspecific hemodynamic effects; followed by 2) AVP (100 pmol/kg; ~ dose of agonist producing 80% of the E_{max}) to assess vasopressive integrity of the animal preparation; 3) a second administration of AVP, also at 100 pmol/kg, to induce an internal (control) reference response; and 4) two doses of FE 202158 (100 and 300 pmol/kg) to identify the equieffective dose to the second administration of the 100 pmol/kg dose of AVP in terms of diastolic arterial pressure (DAP) rise. In time control rats, this was replaced by repeated administration of the 100 pmol/kg dose of AVP. In addition, a control group received an equieffective dose (1000 pmol/kg) of the long-acting selective $V_{1a}R$ agonist F-180 instead of FE 202158. Dosing intervals were set as the time required for the DAP to decrease to a stable baseline (from ~20 min for AVP to ~120 min for F-180).

Antidiuretic Activity in Conscious Rats

The translation of the lack of V_2R agonist activity of FE 202158 in vitro (see *Functional Agonist Activity at the Recombinant Vasopressin and Oxytocin Receptors*) to the in vivo setting was assessed by showing a lack of V_2R-mediated antidiuretic activity in rats compared with the mixed $V_{1a}R/V_2R$ agonist AVP with both compounds infused intravenously at their ED_{50} value for reduction in ear blood flow (i.e., at sizable and equieffective vasoconstrictive infusion rates; see *Vasoconstrictive Activity in Anesthetized Rats*). Specifically, under anesthesia with isoflurane (1–5% in oxygen), female rats preinstrumented by the vendor with a chronic jugular vein catheter had their urinary bladder catheterized through the urethra with the catheter lightly dressed with a topical anesthetic lidocaine/prilocaine (EMLA) cream and fixed in place with tissue surgical cyanoacrylate glue at the level of the urinary meatus. A urine sample was collected immediately after catheterization. Each rat was then allowed to recover from anesthesia (~15 min) while being placed in a restrainer (Experimental Conditioning Unit restrainer; Braintree Scientific, Braintree, MA) over a 37°C heating pad (MUL-T-PAD; Gaymar Industries). The jugular vein catheter was exteriorized from the restrainer and attached to an infusion line from a syringe pump (Genie Plus syringe pump; Kent Scientific) to deliver a hypo-osmotic solution [1.8% (w/v) glucose and 0.24% (w/v) NaCl] at a rate of 500 μl/kg/min (~150 μl/min) to induce substantial water diuresis that reached a steady state after ~2.0 to 2.5 h of infusion (urine osmolality <250 mOsm/kg). A urine sample was collected over the last 20 min of this induction period, and a blood sample was collected at the start of this urine collection for baseline measurements. This steady state was maintained for 110 min during which an infusion of AVP (3.4 pmol/kg/min) or FE 202158 (4.0 pmol/kg/min) was initiated at a

volume rate of 16.7 μl/kg/min (~5 μl/min) for 90 min through the same line as the hypo-osmotic solution. A urine sample was collected over the last 20 min of this infusion period, and a blood sample was collected at the start of this urine collection for measurements of treatment effect. If needed, the hypo-osmotic solution infusion rate was decreased to match any potential treatment-induced decrease in urine flow rate (i.e., antidiuretic effect) at least every 15 min to avoid dilutional hyponatremia. Urine volume was determined gravimetrically (AT261 Delta Range Analytical Balance; Mettler Toledo, Columbus, OH). Urine and plasma osmolality were measured by freezing-point osmometry (model 240 Osmometer; Advanced Instruments, Norwood, MA). Urine sodium and potassium concentrations were determined using the Nova 16 BioProfile Analyzer (Nova Biomedical Corp., Waltham, MA). Plasma sodium and potassium concentrations were determined using the CHEM8+ Cartridge with the i-STAT Handheld Clinical Analyzer (Abbott Laboratories, Princeton, NJ).

Drugs

All chemicals, unless otherwise stated, were of at least U.S.P. grade and used as received from commercial suppliers. All drug doses and concentrations were calculated as free base. [^{3}H]AVP and ^{125}I-ornithine vasotocin were obtained from Perkin-Elmer Life and Analytical Sciences; acetylcholine hydrochloride, nifedipine, sodium nitroprusside, thiobutabarbital sodium (Inactin), and Dibenamine [*N*-(2-chloroethyl)dibenzylamine) hydrochloride] were obtained from Sigma-Aldrich (St. Louis, MO); isoflurane was obtained from Abbott Laboratories; FE 202158, AVP, F-180 (Aurell et al., 1991; Andrés et al., 2002), desmopressin (desamino-[D-Arg^8]vasopressin), OT, carbetocin [carba-1-desamino-[Tyr(Me)^2]oxytocin], and [Pmp^1,Tyr(Me)^2,Arg^8]vasopressin (Kruszynski et al., 1980; Ohlstein and Berkowitz, 1986), were synthesized as acetate salts at Ferring Research Institute, Inc. The chemical structures of FE 202158 and AVP are shown in Fig. 1. For in vitro studies, FE 202158 and AVP were dissolved in dimethyl sulfoxide to give stock solution concentrations of 5 mM and further diluted in a solution of 50 mM Tris-HCl, 5 mM $MgCl_2$, 2.5 mM EDTA, 0.5 mg/ml fatty acid-free bovine serum albumin, pH 7.4 to give a final concentration of dimethyl sulfoxide in microplate wells that was no higher than 0.2% (v/v) in the binding assays or diluted in cell culture media to give a final concentration of 0.1% (v/v) in the reporter gene assays, biologically inactive in vehicle-control groups as verified in separate experiments. For ex vivo studies, the vehicle for FE 202158 and AVP was the modified Krebs physiological salt solution described under *Contractile Activity in the Isolated Rat Common Iliac Artery*. Nifedipine was dissolved in absolute ethanol to give a stock solution concentration of 10 μM and further diluted in modified Krebs physiological salt solution to give a final concentration of ethanol in tissue bath that was no higher than 0.055% (v/v), biologically inactive in vehicle-control groups. Heparin sodium injection U.S.P. (1000 U.S.P. units/ml) was from American Pharmaceutical Partners (Schaumburg, IL), and sterile water, sterile 2.5% and 5% (w/v) dextrose, and 0.45% (w/w) NaCl solution were all from Abbott Laboratories.

Data Analysis

Results are expressed as mean ± S.E.M. or as mean and 95% confidence interval unless otherwise stated. In vitro concentration-response curves were fitted to a four-parameter logistic model using XLfit (IDBS, Almeda, CA), whereas ex vivo concentration-response curves and in vivo dose-response curves were fitted to the three-parameter Hill equation (E_{max} model) using Prism (GraphPad Software Inc., San Diego, CA).

Binding Affinity at the Recombinant Human Vasopressin and Oxytocin Receptors. At each receptor type, the dissociation constant for inhibitor (i.e., test ligand) binding (K_i) values were calculated from the IC_{50} values using the Cheng-Prusoff equation (Cheng and Prusoff, 1973):

$$K_i = IC_{50}/(1 + L^*/K_d) \quad (1)$$

where L^* is the radioligand concentration, and K_d is the radioligand dissociation constant.

pA_2 Determination for the Selective V_{1a}R Antagonist [Pmp^1,Tyr(Me)^2,Arg^8]Vasopressin. The contractile responses to all control and antagonist-treated vascular rings were fitted simultaneously according to the following four-parameter logistic equation using GraphPad Prism, with the unitless Schild slope S set as a constant of one because the antagonist used here is competitive (Ohlstein and Berkowitz, 1986):

$$E = \frac{E_{max}}{1 + \left(\frac{10^{\log EC_{50}}[1 + (B/10^{-pA_2})^S]}{A}\right)^{n_H}} \quad (2)$$

where E is the effect and E_{max} is the maximal possible effect expressed as percentage of control ring maximal contractile response induced by the agonist (FE 202158), EC_{50} is the effective concentration of agonist generating 50% of the maximal possible effect, A is the agonist concentration, B is the antagonist concentration, S is the unitless Schild slope, and n_H is the unitless Hill coefficient. E_{max}, EC_{50}, pA_2, and n_H values are shared between the CCRCs obtained with different concentrations of the antagonist or with its vehicle.

Pharmacokinetics in Conscious Rats. Pharmacokinetic parameters were calculated using noncompartmental curve stripping methods (PK Solutions 2.0; Summit Research Services, Ashland, OH).

Vasopressor Activity in Anesthetized Rats. For each rat, the absolute duration of action of the vasopressor effect of either FE 202158, AVP, or F-180 was calculated as the average of the DAP decay rate, measured over 10-s intervals, from the time of peak DAP increase to the time where 80% of this increase had dissipated (Fig. 2). To normalize for interindividual variation in V_{1a}R agonist responsiveness, the relative duration of action of the vasopressor effect for the dose of FE 202158 (300 pmol/kg) or F-180 (1000 pmol/kg) equieffective to the 100 pmol/kg AVP dose inducing the internal (control) reference response was calculated for each rat as the ratio of the average decay rate for AVP divided by the average decay rate for FE 202158 or F-180 such that a ratio larger than 1 indicates a longer duration of action and a ratio smaller than 1 indicates a shorter duration of action.

Antidiuretic Activity in Conscious Rats. For each rat, free water clearance (C_{H2O}) and effective water clearance (EWC) were calculated according to the following equations (Shoker, 1994; Mallie et al., 1997):

$$C_{H2O} = U_{vol} - (U_{vol} \times U_{osm})/P_{osm} \quad (3)$$

$$EWC = U_{vol} - [U_{vol} \times 2\,(U_{Na} + U_K)]/[2\,(P_{Na} + P_K] \quad (4)$$

where U_{vol} is the urine flow rate in microliters per minute; U_{osm} and P_{osm} are the urine and plasma osmolalities, respectively, both in milliosmoles per kilogram; U_{Na} and P_{Na} are the urine and plasma sodium concentrations, respectively, both millimolar; and U_K and P_K are the urine and plasma potassium concentrations, respectively, both in microliters per minute. EWC is considered a more accurate, although less commonly used, measurement of water clearance than C_{H2O} (Shimizu et al., 2002). EWC can be considered as electrolyte-free water clearance; total electrolytes in urine are approximated here as the sum of Na^+ and K^+ and their accompanying anions. Of relevance to the present study, C_{H2O} overestimates the reduction of free water clearance compared with EWC in the syndrome of inappropriate antidiuretic hormone (AVP) secretion (Mallie et al., 1997). For each of these two variables and U_{osm}, statistical comparison was done using a two-way analysis of variance with repeated measures after an appropriate transformation of the variables such that they

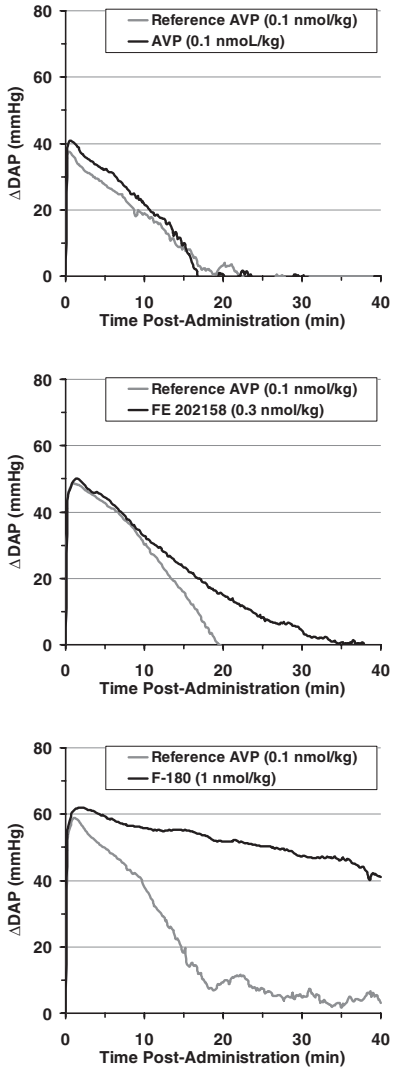

Fig. 2. Diastolic arterial pressure time-response curve in anesthetized Dibenamine-pretreated rats after intravenous bolus injections of AVP (top), FE 202158 (middle), and the long-acting selective $V_{1a}R$ agonist F-180 (bottom). Rats were injected with a reference dose of AVP (100 pmol/kg, intravenous bolus) followed by an equieffective dose of a test compound (FE 202158 at 300 pmol/kg, AVP at 100 pmol/kg, F-180 at 1000 pmol/kg). The mean decay rate of the diastolic pressure increase was determined over the interval between the time of maximal increase and the time where the diastolic arterial pressure increase had decayed by 80% of its maximum.

did not deviate from a normal distribution assumption. The significant analysis of variances ($P < 0.05$) were followed by all pairwise multiple comparison procedures using the Holm Sidak method (SigmaStat v. 3.11; Aspire Software International, Ashburn, VA).

Results

Binding Affinity and Selectivity at the Recombinant Human Vasopressin and Oxytocin Receptors. FE 202158 displayed high affinity and selectivity for the human $V_{1a}R$ compared with the $V_{1b}R$, V_2R, and OTR with a selectivity ratio of >1175:>1739:>190 (ratio of the K_i values; Table 2).

Functional Agonist Activity at the Recombinant Human Vasopressin and Oxytocin Receptors. FE 202158 was a potent and full agonist at the human $V_{1a}R$ in cell-based reporter gene assays (EC_{50} = 2.4 nM, E_{max} = 84%; Table 3). Consistent with the radioligand binding studies, FE 202158 was functionally selective for the human $V_{1a}R$, displaying markedly lower potency and/or efficacy at the $V_{1b}R$ (EC_{50} = 340 nM, E_{max} = 80%), V_2R (EC_{50} = 2656 nM, E_{max} = 66%), and OTR (EC_{50} = 1057 nM, E_{max} = 28%) (selectivity ratio of 1:142:1107:440; Table 3). Likewise, as in the radioligand binding studies, AVP behaved functionally as a nonselective agonist for the human $V_{1a}R$, $V_{1b}R$, V_2R, and OTR with EC_{50} values of 0.24, 4.3, 0.05, and 22 nM (selectivity ratio of 1:18:0.2:92; Table 3).

Functional Agonist Activity at the Recombinant Mouse, Rat, Dog, Sheep, and Pig Vasopressin $V_{1a}R$ and V_2R. FE 202158 was a potent and full agonist at the mouse, rat, dog, sheep, and pig $V_{1a}R$ in cell-based reporter gene assays (EC_{50} values of 18, 0.55, 0.13, 3.8, and 2.2 nM, and E_{max} values of 93, 98, 97, 102, and 92%, respectively; Table 4). As seen for the human receptors, FE 202158 was functionally selective for the $V_{1a}R$ versus the V_2R from the rat, dog, and sheep, and to a lesser extent from the mouse and pig (Table 4). By contrast and consistent with the results at the human receptors, AVP was functionally a relatively nonselective agonist at the $V_{1a}R$ and V_2R in all five animal species tested (Table 4).

Contractile Activity in the Isolated Rat Common Iliac Artery. Consistent with the in vitro results at the rat $V_{1a}R$, FE 202158 was a potent and full contractile agonist (EC_{50} = 3.6 nM, E_{max} = 135% of 80 mM K^+-induced response) compared with AVP (EC_{50} = 0.8 nM, E_{max} = 144% of 80 mM K^+-induced response) in the isolated rat common iliac artery (Fig. 3). The selective $V_{1a}R$ antagonist

TABLE 2
Binding affinity of FE 202158 and AVP at the recombinant human vasopressin and oxytocin receptors

Receptor	K_i		E_{max}		n	Relative Affinity
	Mean	95% CI	Mean	95% CI		
	nM		%			
FE 202158						
$V_{1a}R$	4.4	2.3–9.4	97	95–99	8	1
$V_{1b}R$	>5200		36	25–48	4	>1175
V_2R	>7700		13	0–52	3	>1739
OTR	>840		57	43–72	4	>190
AVP						
$V_{1a}R$	0.3	0.2–0.4	100	100–100	5	1
$V_{1b}R$	1.1	0.8–1.7	100	100–100	4	4
V_2R	3.4	1.1–10.7	100	100–100	3	11
OTR			N.D.			N.D.

N.D., not determined.

TABLE 3
Agonist activity of FE 202158 and AVP at the recombinant human vasopressin and oxytocin receptors

Receptor	EC_{50}			E_{max}		
	Mean	95% CI	*n*	Mean	95% CI	*n*
	nM			%		
FE 202158						
$V_{1a}R$	2.4	1.73–3.35	13	84	67–100	13
$V_{1b}R$	340	130–888	6	80	57–102	6
V_2R	2656	2083–3387	20	66	58–73	20
OTR	1057	269–4153	5	28	23–33	20
AVP						
$V_{1a}R$	0.24	0.22–0.25	608	100*	100–100	616
$V_{1b}R$	4.3	3.9–4.7	347	100*	100–100	360
V_2R	0.05	0.04–0.07	120	95	92–97	137
OTR	22	15–33	5	91	62–120	5

* Efficacy is expressed as E_{max} as a percentage of the relative maximal response to the reference agonist in each assay AVP for $V_{1a}R$ and $V_{1b}R$, desmopressin for V_2R, and carbetocin for OTR.

TABLE 4
Agonist activity of FE 202158 and AVP at the recombinant mouse, rat, dog, sheep, and pig vasopressin and oxytocin receptors

Species	Receptor	EC_{50}			E_{max}		
		Mean	95% CI	*n*	Mean	95% CI	*n*
		nM			%		
FE 202158							
Mouse	$V_{1a}R$	18	11–30	6	93	83–103	6
	V_2R	263	213–324	26	84	79–89	26
Rat	$V_{1a}R$	0.55	0.43–0.70	11	98	89–107	12
	V_2R	447	359–556	25	82	76–88	25
Dog	$V_{1a}R$	0.13	0.09–0.19	5	97	81–112	5
	V_2R	551	369–822	4	78	69–87	4
Sheep	$V_{1a}R$	3.8	1.1–13.0	5	102	87–117	5
	V_2R	489	325–735	8	78	70–85	8
Pig	$V_{1a}R$	2.2	1.0–4.5	5	92	75–109	5
	V_2R	150	84–269	7	76	66–86	7
AVP							
Mouse	$V_{1a}R$	10	9–12	48	100*	100–100	48
	V_2R	0.13	0.10–0.16	10	102	88–115	14
Rat	$V_{1a}R$	0.07	0.06–0.07	282	100*	100–100	305
	V_2R	0.03	0.02–0.04	62	100	97–102	57
Dog	$V_{1a}R$	0.02	0.01–0.03	9	100*	100–100	9
	V_2R	0.39	0.13–1.17	8	103	96–110	7
Sheep	$V_{1a}R$	1.0	0.7–1.4	19	100*	100–100	19
	V2R	0.5	0.3–0.9	5	84	77–91	5
Pig	$V_{1a}R$	1.1	0.7–1.7	21	100*	100–100	21
	V_2R	2.5		2	82		2

* Efficacy is expressed as E_{max} as a percentage of the relative maximal response to the reference agonist in each assay AVP for $V_{1a}R$, desmopressin for V_2R.

[Pmp^1,$Tyr(Me)^2$,Arg^8]vasopressin caused a parallel right shift of the FE 202158 CCRC without reducing the E_{max}, as expected from a competitive antagonist, with a pA_2 of 10.53 (Fig. 4). The contractile response induced by 5.6 nM FE 202158 ($\sim EC_{70}$) was functionally antagonized by the by sodium nitroprusside (IC_{50} = 2.6 nM, E_{max} = 88%; Table 5) and nifedipine (IC_{50} = 4.4 nM, E_{max} = 67%; Table 5).

Vasoconstrictive Activity in Anesthetized Rats. When given by constant intravenous infusion to anesthetized rats, FE 202158 induced a dose-dependent decrease in ear pinna skin blood flow, a surrogate marker for vasoconstriction (ED_{50} = 4.0 pmol/kg/min, E_{max} = 89% reduction from baseline; Fig. 5). Likewise, AVP induced a dose-dependent decrease in ear skin blood flow (ED_{50} = 3.4 pmol/kg/min, E_{max} = 84% reduction from baseline; Fig. 5). At the same time, both compounds produced a dose-dependent increase in systolic and diastolic arterial pressures (results not shown).

Pharmacokinetics in Conscious Rats. To assess the pharmacokinetic behavior of FE 202158 in conscious rats (n = 4), a 3.0 μg/kg dose (2.9 nmol/kg) was injected as a single

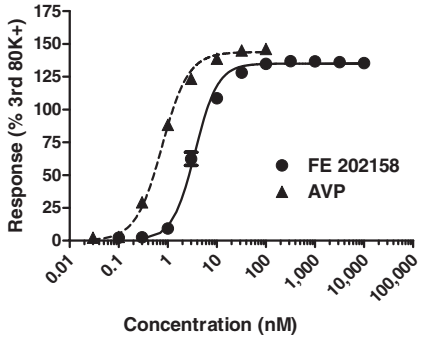

Fig. 3. Concentration-response curves for FE 202158 and AVP ex vivo contractile activity in the rat common iliac artery. EC_{50} mean and 95% confidence interval values in nM were 3.6 (2.9–4.5) for FE 202158 and 0.8 (0.6–1.0) for AVP, E_{max} values in percentage of 80 mM K^+-induced response were 135 (129–141) for FE 202158 and 144 (137–152) for AVP, and unitless n_H values were 1.9 (1.7–2.0) for FE 202158 and 1.6 (1.5–1.7) for AVP. Values are presented as mean ±S.E.M. (S.E.M. bars may be hidden by mean symbols). n = 24 rats for FE 202158 and n = 16 rats for AVP.

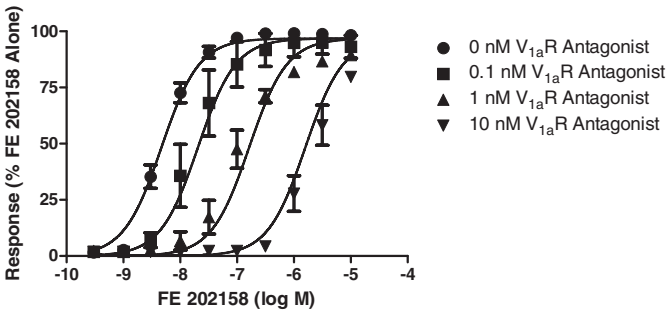

Fig. 4. Concentration-response curves for FE 202158 ex vivo contractile activity in the rat common iliac artery in the presence of increasing concentrations of the selective $V_{1a}R$ antagonist [Pmp^1,$Tyr(Me)^2$,Arg^8]vasopressin. Individual data point and curves obtained by global nonlinear regression are shown. The pA_2 value was 10.5 with a 95% confidence interval of 10.5 to 10.6. Values are presented as mean ± S.E.M. (S.E.M. bars may be hidden by mean symbols). $n = 3$ rats for 0.1 nM, $n = 3$ rats for 1 nM, $n = 4$ rats for 10 nM, and $n = 10$ rats for 0 nM (control contralateral rings).

bolus intravenously. This dose produced plasma concentrations measurable by liquid chromatography-mass spectrometry instrumentation, while the associated peak vasoconstriction was tolerable for the animals. The resulting plasma concentrations (Fig. 6) were well described by a two-compartment model with first-order elimination. The distribution half-life was 3.0 ± 0.5 min, the elimination half-life was 14 ± 0.7 min, the volume of distribution at steady-state was 134 ± 11 ml/kg, and the systemic clearance was 9.4 ± 0.6 ml/kg/min (mean ± S.E.M.).

Vasopressor Activity in Anesthetized Rats. When tested at submaximal (~dose of agonist producing 80% of the E_{max}) equieffective intravenous bolus doses in Dibenamine-pretreated anesthetized rats, both FE 202158 (300 pmol/kg) and AVP (100 pmol/kg) displayed a rapid and time-dependent decrease in the vasopressor response, indicating that FE 202158 is as short-acting as AVP in rats (Fig. 2 and Table 6). At equieffective doses, the vasopressor response relative decay rate for FE 202158 was 1.4 and for time-control AVP it was 1.0. By comparison, in this assay, the vasopressor response relative decay rate for the long-acting selective $V_{1a}R$ agonist F-180 was 5.0 (Fig. 2 and Table 6).

Antidiuretic Activity in Conscious Rats. When given by constant intravenous infusion at the ED_{50} for decrease in ear skin blood flow identified above (Fig. 5) in conscious rats in a state of pronounced water diuresis, AVP induced a marked antidiuretic response, whereas FE 202158 did not induce any antidiuresis (Fig. 7).

Discussion

In this study, we extensively characterized the pharmacological profile of the new peptidic vasopressin analog FE 202158, a novel analog of the mixed $V_{1a}R/V_2R$ agonist hormone AVP, that we engineered to be a potent, selective, and short-acting full $V_{1a}R$ agonist for the treatment of vasodilatory hypotension such as occurs in septic shock. Its short-acting nature should allow dose titration with rapid onset and offset of action by intravenous infusion necessary for the treatment of critical-are indications. Its lack of activity at the V_2R would avoid V_2R-mediated effects observed with AVP that would be deleterious in sepsis, such as water retention through antidiuresis (Knepper, 1997), vasodilation (Kaufmann and Vischer, 2003), and coagulation factor release (Kaufmann and Vischer, 2003).

In binding studies, FE 202158 displayed high affinity and selectivity for the human vasopressin $V_{1a}R$ compared with the $V_{1b}R$, V_2R, and OTR, whereas AVP behaved as a nonselective ligand at the vasopressin receptors. It is noteworthy that in addition to the human $V_{1a}R$, FE 202158 had no significant binding affinity (<25% inhibition of reference compounds binding) at any other molecular target tested (66 G protein-coupled receptors, five ion channels, three transporters), even at high concentration (1 μM) (Cerep, Seattle, WA; results not shown). In contrast, AVP, in addition to its lack of selectivity for the human $V_{1a}R$ compared with the $V_{1b}R$ and V_2R, displayed significant binding to the native κ opioid receptor in guinea pig cerebellum when tested at 1 μM [69% inhibition of reference compound U-50488 ([2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1*R*,2*R*)-2-pyrrolidin-1-ylcyclohexyl]acetamide] binding) (Cerep; result not shown).

This binding selectivity translated in terms of functional activity at the same recombinant human receptors in reporter gene assays; FE 202158 displayed full and potent agonist activity at the $V_{1a}R$, whereas AVP, despite also being a full and potent $V_{1a}R$ agonist, remained functionally nonselective, showing its highest potency at the V_2R. FE 202158 functional potency was deliberately designed to be in the low nanomolar range such that effective plasma concentrations could be measured by liquid chromatography/tandem mass spectrometry; this contrasts with AVP, the subnanomolar potency of which leads to effective plasma concentrations that can be measured only by immunoassay. The mixed $V_{1a}R/V_2R$ agonist character of AVP observed at the human receptors persisted in reporter gene assays at the recombinant mouse, rat, dog, sheep, and pig receptors, whereas FE 202158 maintained its $V_{1a}R$ selectivity versus the V_2R from the rat, dog, and sheep and to a lesser extent from the mouse and pig.

Consistent with the in vitro functional activity results, FE 202158 behaved as a full agonist with a potency of the same order of magnitude as AVP at inducing contraction of the isolated rat common iliac artery ex vivo. This vasoconstrictive effect of FE 202158 was sensitive to the selective $V_{1a}R$ antagonist [Pmp^1,$Tyr(Me)^2$,Arg^8]vasopressin, consistent with the mediation of this effect by the $V_{1a}R$ (Kruszynski et al., 1980; Ohlstein and Berkowitz, 1986). This effect was also partially

TABLE 5
Concentration-response curve fit for sodium nitroprusside and nifedipine against FE 202158 5.6 nM-induced contraction in the isolated rat common iliac artery

Functional Antagonist	IC_{50}		E_{max}		n_H		n
	Mean	95% CI	Mean	95% CI	Mean	95% CI	
	nM		*% inhibition*				
Sodium nitroprusside	2.6	0.8–8.5	88	61–116	1.5	0.5–2.4	3
Nifedipine	5.4	2.0–14.7	59	53–65	1.3	0.9–1.7	14

inhibited by nifedipine and almost fully inhibited by sodium nitroprusside, in accordance with the previously described functional antagonist activity of these agents on AVP-induced contraction in isolated human arteries (Kostrzewska et al., 2000; Wei et al., 2002, 2005; Conant et al., 2003; Kostrzewska et al., 2008). Thus, such antihypertensive drugs would be efficacious "antidotes" in cases of accidental overdosing of FE 202158.

FE 202158 was also a full and potent vasoconstrictor in vivo in rats as shown by its ability to dose-dependently reduce the ear pinna skin blood flow to the same extent as AVP. Taking into account the lower systemic clearance of FE 202158 in the rat (9.4 ml/kg/min) compared with AVP (~50 ml/kg/min) (Crofton et al., 1986), the above ED_{50} values would correspond to EC_{50} values of ~0.43 nM for FE 202158 and ~0.068 nM for AVP. The ear pinna vascular bed is exquisitely sensitive to the vasoconstrictor effect of AVP, being up to 10 times more sensitive than other vascular beds (Garcia-Villalón, 1996), which would explain why both FE 202158 and AVP are ~10 times more potent here than in the isolated common iliac artery.

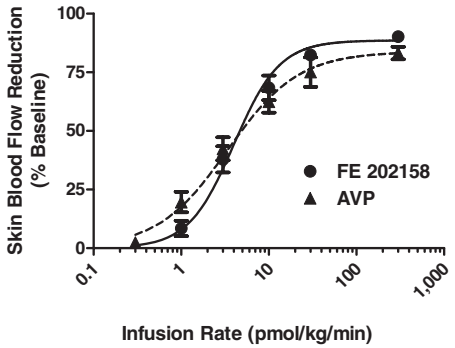

Fig. 5. Dose-dependent reduction of ear skin blood flow from baseline level by intravenous infusion of FE 202158 and AVP in anesthetized rats. ED_{50} mean and 95% confidence interval values in pmol/kg/min were 4.0 (2.1–7.7) for FE 202158 and 3.4 (1.7–6.8) for AVP, E_{max} values in percentage reduction from baseline were 89 (83–94) for FE 202158 and 84 (78–90) for AVP, and unitless n_H values were 1.7 (0.6–2.7) for FE 202158 and 1.1 (0.9–1.3) for AVP. Values are presented as mean ± S.E.M. $n = 4$ rats for FE 202158 and $n = 5$ rats for AVP.

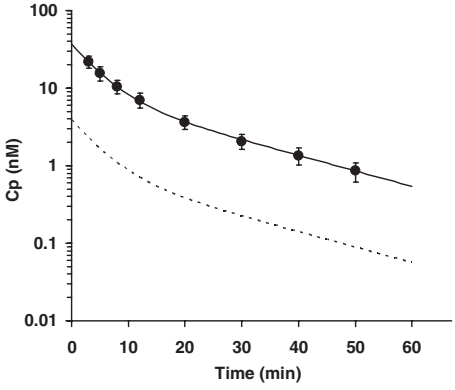

Fig. 6. FE 202158 plasma concentration (Cp) time course in rats after intravenous bolus administration. ●, experimental data at a dose of 3 μg/kg (2.9 nmol/kg) with values presented as mean ± S.D. $n = 4$ rats. Dotted line, predicted plasma concentrations at a dose of 0.3 nmol/kg as used in Fig. 2.

The pharmacokinetics of FE 202158 in rats after intravenous bolus could be well described by a two-compartment model with first-order elimination, suggesting that the vasoconstrictive effect of the dose administered did not have serious detrimental effects on the distribution or elimination of the compound, although the actual values of the resulting pharmacokinetic parameters might have been affected. FE 202158 showed short distribution and elimination half-live values (3.0 and 14 min, respectively) in rats, the same order of magnitude as has been reported previously for AVP (1.06 and 5.96 min, respectively) (Molnár et al., 2007).

The in vivo duration of action of the vasoconstrictive effect of FE 202158 was evaluated using a modification of a classic U.S.P. rat bioassay designed to assess the vasopressor activity of posterior pituitary extracts and synthetic vasopressin analogs (Drucker, 1960). Pretreatment with the irreversible α-adrenergic receptor antagonist Dibenamine eliminates the sympathetic influence on arterial pressure, thereby enhancing the vasopressive effect of intravenous boluses of $V_{1a}R$ agonists, thus making the vasopressive effect a sensitive and specific surrogate marker of the vasoconstrictive effect (Dekanski, 1952). In this assay, FE 202158 was as short-acting as the endogenous hormone AVP unlike the previously disclosed potent and selective $V_{1a}R$ peptidic agonist F-180 (Aurell et al., 1991; Bernadich et al., 1998; Andrés et al., 2002), which exhibited a much longer duration of action. The duration of action of FE 202158 in this model is consistent with the expected plasma concentrations for a compound with low nanomolar potency in vivo. Indeed, the vasoconstrictive effect of FE 202158 almost disappeared by 30 min after administration (Fig. 2), by which time its predicted plasma concentration had decayed to ~0.2 nM (Fig. 6).

Finally, the high $V_{1a}R$ selectivity of FE 202158 versus the V_2R translated into a complete lack of V_2R-mediated antidiuretic effect in a rat model of water diuresis at an intravenous infusion rate corresponding to its ED_{50} for reduction of ear pinna skin blood flow. In contrast, the mixed $V_{1a}R/V_2R$ agonist AVP, at an equieffective vasoconstrictive infusion rate, was markedly antidiuretic. Although the mean values of C_{H2O} and EWC were increased by FE 202158 infusion compared with baseline, these changes were not statistically significant ($P = 0.702$ and 0.868, respectively) and did not represent a consistent increase from rat to rat (C_{H2O} and

TABLE 6
Vasopressor duration of action of FE 202158 and AVP administered as intravenous boluses in anesthetized dibenamine-pretreated rats

Agonist	Diastolic Arterial Pressure Decay Rate		Relative Vasopressor Duration of Action (versus Internal Control Response to AVP)		*n*
	Mean	95% Cl	Mean	95% Cl	
	mm Hg/min				
FE 202158	2.2	−0.1–4.4	1.4	0.9–1.8	3
AVP	2.3	1.8–2.8	1.0	0.8–1.2	9
F-180	0.6	0.4–0.7	5.0	3.4–6.7	7

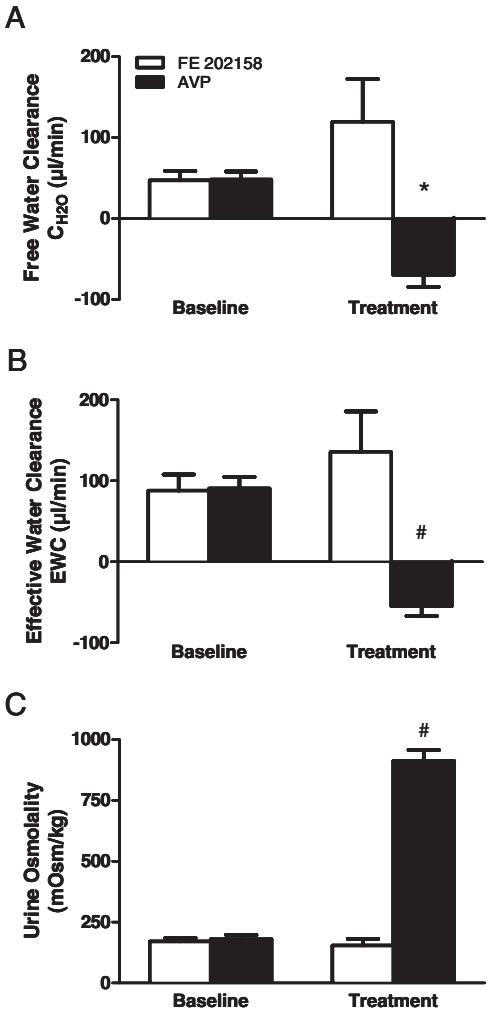

Fig. 7. Effect of FE 202158 and AVP on water diuresis in conscious rats when administered by constant intravenous infusion at the ED_{50} for decrease in ear skin blood (4.0 pmol/kg/min for FE 202158 and 3.4 pmol/kg/min for AVP): C_{H2O} (A), EWC (B), and U_{osm} (C). Values are presented as mean ± S.E.M. $n = 4$ rats for FE 202158 and $n = 6$ rats for AVP. *, $P = 0.001$ versus baseline and $P = 0.002$ versus FE 202158. #, $P < 0.0005$ versus baseline and versus FE 202158.

EWC increased from baseline in only two of four FE 202158-treated rats). Acute increase in arterial pressure, which can be induced by vasopressor compounds such as $V_{1a}R$ agonists, is known to induce a form of osmotic diuresis termed "pressure diuresis" that is driven by natriuresis (Vargas et al., 1997). However, the present assay of intense water diuresis may not be the best model to assess such an effect given that the animals are already in a state of marked diuresis at baseline.

In summary, FE 202158 is a novel, potent, selective, and short-acting peptidic vasopressin $V_{1a}R$ full agonist. This pharmacological profile makes it eminently suitable for the treatment of vasodilatory hypotension occurring in conditions such as septic shock. Indeed, FE 202158 is highly selective for the $V_{1a}R$ unlike the mixed $V_{1a}R/V_2R$ agonist AVP, thus avoiding multiple V_2R-mediated effects such as water retention through antidiuresis (Knepper, 1997), vasodilation (Kaufmann and Vischer, 2003), and coagulation factor release (Kaufmann and Vischer, 2003) that would be deleterious in critical-care medical diseases such as sepsis. In addition, FE 202158 remains potent and short-acting like AVP, allowing for rapid titration of effect by intravenous infusion, including rapid onset and offset of action at reasonable doses/concentrations. We had shown that FE 202158 is superior to AVP in animal models of septic shock; it increased survival in a rat model of platelet-activating factor-induced hypotension, whereas AVP did not (Laporte et al., 2008b) and it reduced vascular leak syndrome to a significantly greater extent than AVP in a sheep model of *Pseudomonas aeruginosa* pneumonia-induced septic shock (Traber, 2007). This compound has thus been selected as a clinical candidate for the treatment of vasodilatory hypotension and is currently undergoing clinical trials in septic shock patients.

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Authorship Contributions

Participated in research design: Laporte, Kohan, Heitzmann, Wiśniewska, Toy, La, Alagarsamy, Ly, Dykert, Qi, Wiśniewski, Croston, Schteingart, and Rivière.

Conducted experiments: Kohan, Heitzmann, Wiśniewska, Toy, La, Ly, Dykert, Wiśniewski, and Galyean.

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Wrote or contributed to the writing of the manuscript: Laporte, Wiśniewska, Toy, La, Alagarsamy, Qi, Wiśniewski, Galyean, Croston, Schteingart, and Rivière.

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