V₂ Receptor Antagonism of DDAVP-Induced Release of Hemostasis Factors in Conscious Dogs


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ABSTRACT

The synthetic arginine vasopressin (AVP) analog 1-desamino-8-D-arginine vasopressin (DDAVP) is used in a variety of hemorrhagic disorders. The present experiments were designed to further characterize the mechanism of DDAVP-induced release of hemostasis factors. The [³H]AVP-labeled AVP receptor in canine renal medullary membranes exhibited an AVP V₂ profile because the V₂ receptor agonist DDAVP displayed similar subnanomolar affinities as the natural hormone AVP, whereas the two selective V₁a compounds SR 49059 and d(CH₂)⁵Tyr(Me)AVP as well as the selective V₁b agonist d-Pal and oxytocin were much less potent. The rank order of the binding affinities of three V₂ receptor antagonists was SR 121463 (a newly described selective V₂ receptor antagonist) > OPC 31260 > d(CH₂)₃[deamino-Cys¹, D-3-(pyridyl 1)-Ala₂,Arg₈]AVP. In conscious dogs, DDAVP (0.1–1 μg/kg IV) caused a dose-related increase (maximum, 43–52% at 30 min) in plasma levels of factor VIII (FVIII), von Willebrand factor (vWF) and tissue-type plasminogen activator (t-PA), but not in levels of plasminogen activator inhibitor-1. A DDAVP-induced hemostasis factor release was also observed in bilaterally nephrectomized dogs. Pretreatment with SR 121463 inhibited DDAVP-induced (1 μg/kg IV) increases in FVIII, vWF and t-PA plasma levels in a dose-dependent manner (ID₅₀ = 14.0 ± 4.0, 12.4 ± 3.0 and 16.7 ± 1.0 μg/kg IV, respectively). OPC 31260 (300 μg/kg IV) revealed a lower activity than SR 121463, and d(CH₂)₃[D-lle²,lle⁴]AVP (30 μg/kg IV) was without effect on the DDAVP response. Pretreatment with SR 49059 (1 mg/kg IV) and SR 27417 (a platelet-activating factor receptor antagonist) (1 mg/kg IV) had no effect on the DDAVP-induced (1 μg/kg IV) increases in FVIII, vWF and t-PA plasma levels. The present results, therefore, strongly suggest that the effect of DDAVP on hemostasis factors occurs via a specific interaction with extrarenal V₂ receptors.

The synthetic AVP analog DDAVP is used in a variety of hemorrhagic disorders. It is used as an antihemorrhagic agent in hemophilia and in von Willebrand disease (Manucci et al., 1977), and it has been used to reduce bleeding side effects caused by various compounds, including aspirin (Flordal and Sahlin, 1993), streptokinase (Johnstone et al., 1984), and it has been used to reduce bleeding side effects caused by various compounds, including aspirin (Flordal and Sahlin, 1993), streptokinase (Johnstone et al., 1984), and it has been used to reduce bleeding side effects caused by various compounds, including aspirin (Flordal and Sahlin, 1993), streptokinase (Johnstone et al., 1984), and it has been used to reduce bleeding side effects caused by various compounds, including aspirin (Flordal and Sahlin, 1993), streptokinase (Johnstone et al., 1984), and it has been used to reduce bleeding side effects caused by various compounds, including aspirin (Flordal and Sahlin, 1993), streptokinase (Johnstone et al., 1984), and it has been used to reduce bleeding side effects caused by various compounds, including aspirin (Flordal and Sahlin, 1993), streptokinase (Johnstone et al., 1984), and it has been used to reduce bleeding side effects caused by various compounds, including aspirin (Flordal and Sahlin, 1993), streptokinase (Johnstone et al., 1984), and it has been used to reduce bleeding side effects caused by various compounds, including aspirin (Flordal and Sahlin, 1993), streptokinase (Johnstone et al., 1984). The synthetic arginine vasopressin (AVP) analog 1-desamino-8-D-arginine vasopressin (DDAVP) is used in a variety of hem-

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ABBREVIATIONS: AVP, arginine vasopressin; DDAVP, 1-desamino-8-D-arginine vasopressin; PAF, platelet-activating factor; FVIII, factor VIII, t-PA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor-1; vWF, von Willebrand factor; BSA, bovine serum albumin; d-Pal, [deamino-Cys¹, D-3-(pyridyl 1)-Ala²,Arg₈]AVP.
induced effects on the coagulation and fibrinolytic systems are lacking. Unlike humans, rats and pigs do not show a DDAVP-induced clotting factor response, but experiments with V2 receptor agonists suggest that dogs and rhesus monkeys may be appropriate (Kinter et al., 1992; Vilhardt and Barth, 1991). The present experiments were designed to further characterize the mechanism of DDAVP-induced release of hemostasis factors in conscious dogs.

Methods

Drugs and dosage. DDAVP, oxytocin, \(d(\text{CH}_3)_2\text{Tyr(Me)}\)-AVP, bacitracin, \(d(\text{CH}_3)_2[\text{l-leu}^2, \text{l-leu}^4]\)AVP and \(\alpha\)-Pal were from Sigma Chemical Co. (Lisle d'Abcoue, France). BSA (type V) was obtained from IBF (Villeneuve La Garenne, France). EDTA, Tris and dimethylsulfoxide were from Merck-Clevenot (Nogent sur Marne, France). [\(^{3}H\)AVP (80 Ci/mmol) was purchased from New England Nuclear (Les Ulis, France). The V2 receptor antagonist \(d(\text{CH}_3)_2[\text{l-leu}^2, \text{l-leu}^4]\)AVP and SR 121463 were dissolved in saline. SR 27417 and OPC 31260 receptor antagonist SR 49059 was dissolved in a solution containing ethanol, H\(_2\)O, glycerol and polyethylene glycol (60:30:5:5 v/v). SR 27417 (Herbert et al., 1991) was from Sanofi Recherche (Toulouse, France). For the in vivo studies, DDAVP, \(d(\text{CH}_3)_2[\text{l-leu}^2, \text{l-leu}^4]\)AVP and SR 121463 were dissolved in saline. SR 49059 was dissolved in a solution containing ethanol, H\(_2\)O, glycerol and polyethylene glycol (60:30:5:5 v/v). SR 27417 and OPC 31260 were solubilized in 0.1 N HCl in saline (1:40 v/v). All substances were administered intravenously as solutions prepared daily before the administration. Control dogs were treated with saline. For in vitro binding experiments, SR 121463, SR 49059 and OPC-31260 were dissolved in dimethylsulfoxide (10\(^{-2}\) M) and then diluted in the test solvent.

Membrane preparations. Both kidneys from pentobarbital-anesthetized male mongrel dogs were chilled in ice-cold saline. The renomedullary regions, which constitutively express AVP V2 receptors, were immediately dissected. Membranes were prepared according to the method of Stassen et al. (1982) as described recently (Serradeil-Le Gal et al., 1996) and stored as aliquots in liquid nitrogen at a final concentration of ~10 mg of protein/ml. Protein concentration was determined according to Bradford (1976) with BSA as a standard.

AVP \(V_2\) binding assay. Renomedullary membranes (100–150 \(\mu\)g/assay) were incubated for 45 min at 25°C in a 50 mM Tris-HCl buffer; pH 8.1, containing 2 mM MgCl\(_2\), 1 mM EDTA, 0.1% BSA, 0.1% bacitracin, 3 nM [\(^{3}H\)AVP and increasing amounts of the test compounds. The reaction was stopped by the addition of 4 ml of ice-cold buffer followed by filtration through GF/B Whatman glass microfiber filters. Filters were washed twice with 4 ml of ice-cold buffer and counted by liquid scintillation using a beta scintillation counter (Packard, Tricarb). Saturation experiments were performed with increasing concentrations of [\(^{3}H\)AVP (0.03–15 nM). Nonspecific binding was determined by incubation with 1 \(\mu\)M AVP. Data for equilibrium binding (\(K_d\), apparent equilibrium dissociation constant, and \(B_{max}\), maximum binding density) and competition experiments (IC\(_{50}\), \(nM\)) were analyzed by an iterative nonlinear regression program using the software RS.1 (Munson and Rodbard, 1980). The IC\(_{50}\) value was defined as the concentration of inhibitor required to obtain 50% inhibition of the specific binding. Inhibition constant (\(K_i\)) values were calculated from the IC\(_{50}\) values using the Cheng and Prusoff equation (1973).

Animals and procedures. Twelve-month-old male mongrel dogs weighing 18 to 26 kg were used. The animals were fed a standard laboratory chow (Doko, Fontaine-les-Vervins, France), and tap water was available ad libitum. Before the experiments, the dogs were fasted overnight. They were trained to stand quietly on a table. Blood (4 ml) was collected through venipuncture of cephalic veins immediately before and at indicated intervals after intravenous injections of DDAVP in tubes containing trisodium citrate (3.8%, 1/9 v/v) for measurements of vWF, FVIII and PAI-1 plasma concentrations. At \(<\)2 min after withdrawal, 300 \(\mu\)l of sodium acetate (0.2 M) was added to 300 \(\mu\)l of blood for determination of t-PA activity. Blood samples were immediately centrifuged at 4°C (1000 \(\times\) g for 15 min). The plasma was kept at −80°C until use. Antagonists were injected intravenously 5 min before administration of DDAVP. Some dogs repeated the protocol with a minimum interval of 1 week between studies. In these dogs, no tolerance to DDAVP-induced release of hemostasis factors was observed.

In another series of experiments, dogs were anesthetized with pentobarbital (30 mg/kg i.p.), and both kidneys were removed. Blood collections after DDAVP injection to the anesthetized dogs were performed as described above.

The protocol of this study has been approved by the Animal Care and Use Committee of Sanofi Recherche, Toulouse.

Analytical methods. FVIII (VIII:C) levels in plasma were measured using an immunodepleted plasma from Diagnostica Stago (Asnières, France). vWF was measured by means of an enzyme immunosorbent assay procedure with the Asserachrom vWF kit (Diagnostica Stago, Asnières, France). Activity of t-PA was determined with Coasert t-PA by measuring the amidolytic activity of plasmin from the chromogenic substrate S-2251. The PAI plasma activity was determined with Coaest PAI (Chromogenix, Molndal, Sweden) by measuring the plasmin formed from plasminogen in the presence of t-PA from the chromogenic substrate S-2403.

Data analysis. Results are expressed as mean ± S.E.M. Statistical analyses were performed using the Mann-Whitney U test, and \(P < 0.05\) was accepted as a significant difference. ID\(_{50}\) values were calculated by fitting the logistic equations to the data by linear regression.

Results

Binding studies. AVP receptors in canine renomedullary membranes were characterized using [\(^{3}H\)AVP as a ligand. Saturation experiments, performed with increasing concentrations of [\(^{3}H\)AVP, showed that the specific binding was saturable. Scatchard analysis of the data (fig. 1) gave a linear plot consistent with the presence of a single class of high-affinity binding sites with a \(K_d\) and a \(B_{max}\) value of 0.32 ± 0.03 nM and 100 ± 4 fmol/mg of protein, respectively. A

![Fig. 1. Scatchard plot of [\(^{3}H\)AVP binding to canine renomedullary membranes. Saturation experiments were conducted in the presence of increasing concentrations of [\(^{3}H\)AVP (0.03–15 nM) and 140 \(\mu\)g/assay membranes as described in the text. Results shown are the mean of three experiments performed in triplicate.](image-url)
similar affinity has been reported with [3H]AVP in kidney preparations of rat, bovine and human origin (Manning et al., 1984; Serradeil-Le Gal et al., 1996; Yamamura et al., 1992).

The binding of [3H]AVP was further characterized by studying the relative affinities of several reference peptide or nonpeptide AVP/oxytocin compounds (table 1). The labeled receptor exhibited a AVP V2 profile because the V2 receptor agonist DDAVP and the natural hormone AVP displayed similar subnanomolar affinities, whereas the two selective V1a compounds SR 49059 and d(CH2)6Tyr(Me)-AVP as well as the selective V1b agonist d-Pal (Schwartz et al., 1991) and oxytocin were much less potent.

The nonpeptide AVP V2 receptor agonist SR 121463 competed with high potency at dog kidney V2 receptors (table 1) and displayed an affinity for this receptor in good agreement with those previously obtained in rat, bovine and human remodellable membranes (K i = 1.42, 0.64 and 4.1 nM, respectively) (Serradeil-Le Gal et al., 1996). OPC-31260 and d(CH2)3[de2,ile4]AVP were much less potent than SR 121463 (<30- and 170-fold, respectively) at canine remodellable AVP V2 receptors (table 1). It is important to note that dose-response displacement curves for all compounds tested in this study gave linear Hill plots and pseudo-Hill coefficients (nH) of −1 (not shown), indicating competitive antagonism with the ligand.

**DDAVP-induced release of hemostasis factors.** In conscious dogs, initial plasma concentrations of FVIII and vWF immediately before DDAVP treatment amounted to 0.4 IU/ml and 32.0 arbitrary units/ml, respectively (n = 16). In vehicle-treated controls, plasma levels of these four parameters were stable for the duration of the study (4 hr, n = 3). As shown in figure 2, DDAVP (0.1–1 μg/kg i.v.) induced a dose-related increase in plasma levels of FVIII, vWF and t-PA as measured 30 min after DDAVP administration. The time courses of the variations of FVIII, vWF and t-PA plasma levels after a single intravenous administration of 1 μg/kg DDAVP are illustrated in figure 3 (controls). Maximum increases in FVIII (43%), vWF (52%) and t-PA (50%) plasma levels were observed at 30 min after DDAVP administration. Plasma concentrations of FVIII returned to base-line values in a linear fashion within 4 hr. The decrease in vWF plasma concentrations was delayed. As a consequence, elevated vWF concentrations were still observed 4 hr after DDAVP administration (22%, P < .05). Plasma activity of t-PA returned to pretreatment values within 1 hr and tended to be lower than pretreatment values at 2 and 4 hr (−14% and −21%, P > .05). No significant changes in PAI-1 plasma activity were recorded during the 4-hr observation period (data not shown).

In bilaterally nephrectomized dogs, DDAVP also produced a hemostasis factor response. After intravenous administration of DDAVP (1 μg/kg), FVIII, vWF and t-PA plasma levels were increased to 231 ± 40%, 183 ± 40% and 209 ± 18%, respectively, of the pretreatment values (n = 3).

**Influence of V1a and V2 receptor antagonism on DDAVP effects.** Pretreatment with the V2 receptor antagonist SR 121463 inhibited DDAVP-induced (1 μg/kg i.v.) increases in FVIII, vWF and t-PA plasma levels in a dose-dependent manner (fig. 3). ID50 values for inhibition of DDAVP-induced increases in FVIII, vWF and t-PA at 30 min were 14.0 ± 4.0, 12.4 ± 3.0 and 16.7 ± 1.0 μg/kg i.v., respectively. Increases in plasma concentrations of these three proteins after DDAVP injection were completely eliminated at 100 μg/kg SR 121463 i.v.

Figure 4 compares the effects of the three V2 receptor antagonists SR 121463, OPC 31260 and d(CH2)5[de2,ile4]AVP on increases in FVIII plasma concentrations after administration of DDAVP (1 μg/kg i.v.). OPC 31260 revealed a lower activity than SR 121463. At an OPC 31260

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**TABLE 1**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Ki (nM)</th>
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<tr>
<td>AVP</td>
<td>0.31 ± 0.04</td>
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<tr>
<td>DDAVP</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td>d-Pal</td>
<td>50 ± 11</td>
</tr>
<tr>
<td>d(CH2)3de2,ile4AVP</td>
<td>122 ± 11</td>
</tr>
<tr>
<td>d(CH2)3Tyr(Me)-AVP</td>
<td>251 ± 5</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>122 ± 7</td>
</tr>
<tr>
<td>Nonpeptide</td>
<td></td>
</tr>
<tr>
<td>SR 121463</td>
<td>0.70 ± 0.10</td>
</tr>
<tr>
<td>OPC 31260</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>SR 49059</td>
<td>258 ± 20</td>
</tr>
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</table>

Inhibition constants (Ki) were calculated according to Cheng and Prusoff (1973). Values are mean ± S.E.M. (n = 6).
dose of 300 μg/kg i.v., DDAVP-induced increases in FVIII plasma concentrations were inhibited by only 57%. As can be seen in figure 4, pretreatment with d(CH2)5[D-lle2,lle4]AVP at 30 μg/kg i.v. did not reveal any effect on the DDAVP FVIII response. Similarly, d(CH2)5[D-lle2,lle4]AVP (30 μg/kg i.v.) did not inhibit DDAVP-induced (1 μg/kg i.v.) increases in vWF and t-PA plasma concentrations, whereas OPC 31260, at 300 μg/kg i.v., exhibited a moderate inhibitory activity of 42% and 49%, respectively, on these parameters (n = 3 in the control group). 100% refers to the values measured before DDAVP administration. For clarity, S.E.M. bars are presented for the saline-treated group only. * P < .05 compared with the groups treated with saline.

This dose, SR 49059 alone did not influence the measured parameters during the 4-hr observation period.

**Influence of PAF receptor antagonism on DDAVP effects.** Another series of in vivo experiments was performed to examine whether PAF was involved in the DDAVP-induced release of hemostasis factors as suggested by Hashemi et al. (1993). At 5 min before DDAVP (1 μg/kg i.v.) administration, dogs were pretreated i.v. with 1 mg/kg of the PAF receptor antagonist SR 27417. As can be seen in figure 5, SR 27417 pretreatment did not modify DDAVP-induced increases in FVIII, vWF and t-PA levels in plasma. At this high dose, SR 27417 alone did not influence the measured parameters during the 4-hr observation period.

**Discussion**

The present results confirm that the intravenous administration of DDAVP induces a release of FVIII, vWF and t-PA in conscious dogs. Comparable increases in plasma levels of these proteins with similar kinetics have been previously described in conscious dogs (Vilhardt and Barth, 1991) and anesthetized monkeys (Kinter et al., 1992), whereas humans are more sensitive to DDAVP-induced release of vWF and t-PA (Mannucci et al., 1975). The possible influence of DDAVP on PAI-1 plasma activity has not previously been reported. Present results in conscious dogs do not demonstrate evidence of a involvement of PAI-1 in the effect of DDAVP to release hemostasis factors.

SR 121463 and OPC 31260, two novel, selective nonpeptide antagonists for V2 receptors, possess a high affinity for renal V2 receptors and inhibit AVP-induced cAMP formation (Manning et al., 1984; Serradeil-Le Gal et al., 1996; Yamamura et al., 1992). Both compounds also revealed potent in vivo activities: OPC 31260 exerted an aquaretic effect in rats, dogs,
monkeys and humans, and SR 121463 showed aquaretic activity in rats (Fujisawa et al., 1993; Serradeil-Le Gal et al., 1996; Yamamura et al., 1992). In the present study, SR 121463 inhibited DDAVP-induced releases of FVIII and vWF in a dose-dependent manner with ID50 values of 14.0 ± 4.0 and 12.4 ± 3.0 μg/kg i.v., respectively. OPC 31260 revealed a lower activity than SR 121463 in inhibiting DDAVP-induced clotting factor release. In addition, the present study is the first demonstration of an inhibition of DDAVP-induced t-PA release via V2 receptor antagonism.

The inhibitory actions of SR 121463 and OPC 31260 on the DDAVP-induced clotting factor response are in agreement with observations of Kinter et al. (1992), who reported total prevention of DDAVP-induced clotting factor release by the peptide V2 receptor antagonist SK&F 105494 in rhesus monkeys. Contrary to this, Vilhardt and Barth (1991) reported failure to block DDAVP-induced release of FVIII and t-PA by the V2 receptor antagonist d(CH2)5[D-lle2,lle4]AVP in dogs and concluded that the ability of DDAVP to release FVIII and t-PA does not involve V2 receptors. This conclusion, however, must be considered premature. Despite close structural similarities between AVP V2 receptors cloned in several species such as rat, pig, cow and humans (Lolait et al., 1994; Birnbaumer et al., 1992; Gorbulev et al., 1993), marked interspecies differences exist for AVP V2 receptors on the basis of affinity and efficacy of certain AVP analogs (Ufer et al., 1995; Guillem et al., 1982). Peptide V2 receptor antagonists with branched side-chain amino acid substitutions at positions 2 and 4, such as d(CH2)5[D-lle2,lle4]AVP, show substantial interspecies variability in affinity for renal V2 receptors; they are inactive as antidiuretic antagonists in dogs (Kinter et al., 1992). In line with these findings are the present in vivo observations that d(CH2)5[D-lle2,lle4]AVP did not reveal an inhibitory effect on DDAVP-induced release of hemostasis factors. These data suggest a low affinity of d(CH2)5[D-lle2,lle4]AVP to canine V2 receptors.

The present in vitro results with canine renomedullary membrane preparations confirm this conclusion. The affinity of d(CH2)5[D-lle2,lle4]AVP was more than 2 orders of magnitude lower than that of SR 121463. d(CH2)5[D-lle2,lle4]AVP strongly discriminates between dog and rat V2 receptors because this compound, which is well known for its potent antivasopressin diuretic properties in rats (pA2 = 8.04), was found in this study to be ~100-fold less potent at dog (Kd = 122 nM) than at rat (Kd = 1.1 nM) V2 receptors (Manning et al., 1984). The present in vitro findings also give a good explanation for the differences in in vivo activity between SR 121463 and OPC 31260 to inhibit the release of hemostasis factors. The observed affinity of OPC 31260 in this study is in agreement with previous results obtained in rat, bovine and human preparations (Serradeil-Le Gal et al., 1996).

Although the agonist activity of DDAVP is generally considered to be V2 mediated, DDAVP also possesses V1a and V3 receptor agonist activity (Wun et al., 1995; Ammar et al., 1994). A V1a receptor-mediated mechanism has been described for DDAVP-induced platelet activation (Wun et al., 1995). In the current model, however, the V1a receptor antagonist SR 49059 had no effect on the DDAVP-induced increases in FVIII, vWF and t-PA plasma levels. Taken together, these data strongly suggest that the stimulation of extrarenal V2-like receptors is the main mechanism involved in the DDAVP-induced release of FVIII, vWF and t-PA. This conclusion is also in agreement with the clinical findings that male patients with congenital X chromosome-linked nephrogenic diabetes insipidus do not exhibit a rise in FVIII plasma concentrations when treated with DDAVP (Kobrinsky et al., 1985). Diuresis in these patients is due to mutation of renal V2 receptors; perhaps the missing DDAVP-clotting factor response is due to the same mutation of extrarenal V2-like receptors. There is no evidence as yet to the location of the V2 AVP receptors that are involved in the DDAVP response and intracellular signaling mechanisms. Because bilateral nephrectomy did not prevent the effect of DDAVP in the present experiments, it appears that the receptors responsible for the increase in hemostasis factors are extrarenal. Further evidence for the existence of extrarenal V2 receptors has been previously provided. The DDAVP-induced releases of cAMP
in anephric dogs and of hemostasis factors in surgically anephric patients were not different from those in the control groups with intact kidneys (Liard, 1992; Mannucci et al., 1975). It is tempting to speculate that the extrarenal V2 receptors accounting for the DDAVP-induced increase in hemostasis factors might be on endothelial cells; however, there is no proof of the existence of V2 receptors on endothelial cells.

The involvement of PAF in the DDAVP-clotting factor response has been postulated by Hashemi et al. (1993). Their in vitro findings indicated that the stimulation of endothelial cells by DDAVP may be an indirect effect mediated through stimulation of a monocyte V2 receptor. In turn, PAF would be secreted by DDAVP-treated monocytes and enhance the release of vWF from endothelial cells. Present in vivo experiments with the PAF receptor antagonist SR 27417, however, do not indicate an involvement of such a mechanism in the DDAVP-induced release of vWF or in the release of FVIII and t-PA.

In summary, the present in vivo results in conscious dogs, together with the in vitro findings in canine renomedullary membranes, strongly support the conclusion that the effect of DDAVP on hemostasis factors occurs via a specific interaction with extrarenal V2 or V2-like receptors, whose localization remains to be further explored. The present data argue that the effect of DDAVP does not involve PAF or V1a receptors.

References


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