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REVIEW ARTICLE

Cellular mechanisms of the hemostatic effects of desmopressin (DDAVP)

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Please see also Cash J. D. DDAVP and factor VIII: a tale from Edinburgh. This issue, pp. 619–621. Mannucci P. M. Desmopressin (DDAVP) and factor VIII: the tale as viewed from Milan (and Malmö). This issue, pp. 622–624. Kaufmann J. E. *et al*. Desmopressin (DDAVP) induces NO production in human endothelial cells via V2 receptor- and cAMP-mediated signaling. This issue, pp. 821–828.

Summary. The synthetic analog of vasopressin desmopressin (DDAVP) is widely used for the treatment of patients with von Willebrand disease (VWD), hemophilia A, several platelet disorders, and uremic bleeding. DDAVP induces an increase in plasma levels of von Willebrand factor (VWF), coagulation factor VIII (FVIII), and tissue plasminogen activator (t-PA). It also has a vasodilatory action. In spite of its extensive clinical use, its cellular mechanism of action remains incompletely understood. Its effect on VWF and t-PA as well as its vasodilatory effect are likely explained by a direct action on the endothelium, via activation of endothelial vasopressin V2R receptor and cAMP-mediated signaling. This leads to exocytosis from Weibel Palade bodies where both VWF and t-PA are stored, as well as to nitric oxide (NO) production via activation of endothelial NO synthase. The mechanism of action of DDAVP on FVIII plasma levels remains to be elucidated. The hemostatic effect of DDAVP likely involves additional cellular effects that remain to be discovered.

Keywords: desmopressin, endothelium, vasopressin V2 receptor.

Introduction

Desmopressin (DDAVP), a synthetic analog of vasopressin (AVP), has been used for almost 25 years for the treatment of von Willebrand disease (VWD) and other bleeding disorders. The antidiuretic hormone AVP was first shown to elevate circulating levels of coagulation factor VIII (FVIII) by Mannucci *et al*. in 1972 [1]. However, this was accompanied by side-effects, preventing its use as a therapeutic agent. A few years later, Cash *et al*. [2] and Mannucci *et al*. [3] showed that its synthetic analog DDAVP, first used to treat nephrogenic diabetes insipidus (NDI) [4,5], also increased plasma levels of FVIII, von Willebrand factor (VWF) and plasminogen activator activity. In addition, this substance was shown to possess little or no pressor activity. DDAVP has been shown to rapidly shorten the bleeding time and to reduce blood loss in patients with mild and moderate hemophilia A, selected types of VWD, several forms of platelet dysfunction and uremic bleeding [6]. The use of DDAVP rather than blood-derived concentrates has allowed for a reduced risk of transfusion-transmitted diseases [7].

In spite of its extensive clinical use, the mechanisms of action of DDAVP have long remained unknown. The purpose of this paper is to review recent progress on the mechanisms of DDAVP on VWF, t-PA and FVIII levels, as well as DDAVP-induced vasodilation. We will also highlight the several aspects of DDAVP physiology that remain poorly understood.

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Effect of DDAVP on VWF release

Plasma VWF derives from endothelial secretion

VWF is a large glycoprotein playing a role in primary hemostasis, by mediating adhesion of platelets to the subendothelium. It also functions as a carrier protein for FVIII, protecting it from proteolytic degradation. The structure and function of VWF has been largely reviewed [8–10]. VWF is synthesized in endothelial cells (EC) and megakaryocytes as a precursor, pro-VWF. This precursor undergoes dimerization, glycosylation, proteolytic cleavage into VWF and a propeptide (VWFAg:II) and assembly of the dimers into large multimers (500–15 000 kDa). Multimerized VWF, together with equimolar amounts of propeptide, is stored in specialized secretory granules called Weibel-Palade bodies (WPB). VWF is rapidly released from WPBs by exocytosis in response to a variety of secretion agonists. (pro-) VWF is synthesized by both ECs and megakaryocytes, but plasma VWF appears to be mainly of endothelial origin [11]. *In vivo*, DDAVP induces the release of highly multimerized forms of VWF [12] (which are the most efficient forms for its role in primary hemostasis [13]). Therefore, the DDAVP-induced rise in circulating plasma VWF most likely reflects direct release of VWF from WBP in ECs. This conclusion is confirmed by the observation that DDAVP induces an equimolar increase in VWF and propeptide plasma levels [14,15].

General mechanisms of regulated endothelial VWF secretion

Acute VWF release from WPBs in cultured human umbilical vein endothelial cells (HUVECs) is induced by receptor agonists, such as thrombin and histamine, that act via a rise in cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) [16–18]. However, most of these agonists are mediators of inflammation and/or thrombosis, acting in a local or regional manner. They are therefore unlikely to participate in the physiological regulation of plasma VWF levels. cAMP-raising agents including forskolin (an activator of adenylyl cyclase), epinephrine, adenosine and prostacyclin also induce VWF secretion in HUVECs, independent of a rise in $[Ca^{2+}]_i$ [19–22]. In contrast to thrombin and histamine, which cause EC contraction, cAMP-raising agents preserve cell-cell contacts in endothelial monolayers [23]. cAMP-raising agents likely regulate the physiological variations in plasma VWF levels. Indeed, epinephrine infusion raises plasma VWF levels, and physical activity increases plasma VWF levels via catecholamines acting on β2-adrenergic, adenylyl cyclase-coupled receptors [24,25].

DDAVP induces direct exocytosis from ECs

In contrast to AVP, which binds to three cellular receptors (V1a, V1b, V2), DDAVP is a selective agonist for the V2 receptor (V2R). This vasopressin receptor subtype is expressed in the kidney collecting duct, and mediates the antidiuretic effect of the hormone [26,27]. DDAVP fails to cause an increase in circulating VWF in patients with diabetes insipidus who possess a mutation in the V2R [28–30]. However, in patients with chronic renal failure, DDAVP raises plasma VWF even after bilateral nephrectomy [3]. These clinical observations suggest the involvement of extrarenal V2R. The simplest hypothesis then becomes that DDAVP directly binds to endothelial V2R, inducing rapid VWF secretion from WPBs.

In the kidney collecting duct, AVP or DDAVP activating the V2R cause water retention by inducing the translocation of the water channel aquaporin-2 from intracellular stores to the apical plasma membrane, an example of cAMP-mediated exocytosis [31]. Assuming a similar signaling pathway, we studied DDAVP-induced, cAMP-mediated VWF secretion in cultured human ECs. HUVECs do not express V2R. However, we reported a DDAVP-mediated increase in VWF secretion after heterologous expression of V2R in these cells [32]. Moreover, we showed DDAVP-induced, cAMP-mediated VWF secretion in primary human microvascular ECs of the lung (HMVEC-L). We demonstrated V2R expression in HMVEC-L as well as in whole human lung, by RT-PCR [32]. These data provide strong support for the hypothesis that DDAVP-induced VWF secretion results from V2R-mediated, cAMP-dependent exocytosis from WPBs. We have therefore demonstrated functional V2R expression in the endothelium and provided a cellular mechanism for the effects of DDAVP on VWF plasma levels.

Several authors have proposed an indirect mechanism for DDAVP-induced VWF secretion: DDAVP activates an intermediate cell, which in turn secretes a VWF-releasing hormone that acts on ECs [6,33]. One proposed intermediate cell was in the hypothalamus, but this notion has now been disproved [34,35]. Hashemi *et al*. have suggested that platelet-activating factor (PAF) released from monocytes in response to DDAVP induces VWF secretion from ECs [33]. However, these authors have not demonstrated V2R expression in monocytes and others could not detect V2R expression in these cells [36]. The observation that pretreatment with an inhibitor of the PAF receptor does not suppress DDAVP-induced VWF secretion in conscious dogs also argues against this hypothesis [37]. Previous difficulties with the hypothesis of a direct effect of DDAVP on ECs were that V2R expression, studied by molecular techniques, is limited to the kidney [26]. Further, exposure of cultured ECs to DDAVP failed to induce VWF release [19,38,39]. We now have shown that V2R is expressed in some ECs other than HUVECs.

DDAVP and t-PA release

The profibrinolytic role of DDAVP was one of its first effects to be described [2,3]. This profibrinolytic activity is due to an increase in tissue plasminogen activator (t-PA). t-PA converts plasminogen to plasmin and thus initiates fibrin degradation, an activity which is enhanced by fibrin binding (reviewed in [40]).

T-PA is stored and released from ECs

The vascular endothelium is thought to be the main source of plasma t-PA [41]. In cultured ECs, t-PA is expressed at low levels. Its synthesis is up-regulated, usually at the transcriptional level, in response to fluid shear stress, thrombin, histamine, retinoic acid, VEGF and sodium butyrate [42–46]. In addition, there is both *in vivo* and *in vitro* evidence that t-PA is acutely released from preformed stores. A rapid increase in plasma t-PA levels is observed in response to DDAVP, as well as β-adrenergic agents, administered systemically or in forearm perfusion studies [47,48]. In HUVECs, acute t-PA release has been shown in response to $[Ca^{2+}]_i$-raising agents such as thrombin, and cAMP-raising agents such as epinephrine and Iloprost [22].

T-PA and VWF costorage in WPB

Colocalization of VWF and t-PA to the same compartment could account for the coordinate effect of DDAVP on the plasma level of the two proteins. In our hands t-PA is only expressed at low levels in HUVECs. However, various pretreaments (VEGF, retinoic acid or sodium butyrate) induced an up-regulation of t-PA synthesis. t-PA was exclusively located to WPBs, as observed by both immunofluorescence and immunoelectron microscopy [49]. Identification of t-PA in WPBs in HUVECs has also been reported by Datta *et al*. [50]. Incubation with histamine, forskolin and epinephrine induced the rapid, coordinate release of both t-PA and VWF, consistent with a single storage compartment [49]. We attempted to demonstrate DDAVP-induced t-PA release from HMVEC-L cells, however, these cells

did not express t-PA, even after various pretreatments (authors' unpublished observations). In native human skeletal muscle, t-PA was expressed in ECs from arterioles and venules, along with VWF. The two proteins were found to be colocalized in WPBs by immunoelectron microscopy [49]. These data indicate that t-PA and VWF are colocalized in WPBs, both in HUVECs and *in vivo*. Thus, release of t-PA and VWF from the same storage pool accounts for the coordinate increase in the plasma level of the two proteins in response to DDAVP and other stimuli.

Is there an additional t-PA storage compartment?

The identification of the endothelial storage pool and the mechanism controlling the regulated secretion of t-PA have been the topic of numerous studies. Emeis *et al*. have suggested a storage compartment for t-PA, distinct from WPBs [51]. In cell fractionation experiments of rat lung homogenates, these authors found t-PA at a different density than VWF on sucrose gradients, although t-PA and VWF migrated at the same density on Nycodenz gradients. Using both immunofluorescence and immunoelectron microscopy in HUVECs, they identified t-PA in small, round vesicles. These authors also identified subtle differences in the kinetics of release of t-PA and VWF from HUVECs, leading them to propose a t-PA storage compartment distinct from WPBs. The discrepancy with the findings of Emeis *et al*. remains unexplained, although they are possibly accounted by differences in cell culture conditions.

In human forearm perfusion studies, t-PA release was induced by DDAVP and bradykinin among other agonists [47,52]. However, contrary to our prediction, DDAVP failed to induce coordinate VWF release [47], possibly indicating a distinct mechanism of storage and release of t-PA and VWF. Thus, DDAVP appears to induce VWF secretion after intravenous injection but not after perfusion into the brachial artery. This discrepancy in t-PA and VWF secretion is possibly explained by analytical considerations. In forearm perfusion studies, net t-PA release is calculated as the product of the arterio-venous difference in t-PA concentration and blood flow. Since DDAVP also increases blood flow, significant increases in t-PA release may translate into fairly small increases in the arterio-venous difference in t-PA concentration. VWF has a much longer half-life than t-PA (6–12 h vs. <5 min), accounting in part for a higher basal circulating level (approx. $10\,\mu g\,mL^{-1}$ vs. $5–10\,ng\,mL^{-1}$) [47]. It is therefore quite likely that after an acute stimulus, a smaller relative increase in the arterio-venous difference in VWF levels would have escaped detection. It is also worth noting that the relative level of expression of t-PA and VWF has not been studied in detail in different human organs and vessel types. A high t-PA/VWF ratio in skeletal muscle may contribute to the apparent increase in t-PA but not in VWF release after DDAVP in forearm perfusion studies.

DDAVP-induced vasodilation

DDAVP is known to have vasodilator properties, as shown by an increase in heart rate and a decrease in systolic and diastolic blood pressure, as well as facial flushing [29,53]. These effects are not observed in patients with NDI [29], suggesting an involvement of V2R. Forearm perfusion studies have demonstrated that AVP or DDAVP exert a direct vasodilatory effect after intra-arterial administration, in a nitric oxide (NO)-dependent manner [54–56]. These observations suggest a direct, local activation of endothelial NO synthase (eNOS) in the skeletal muscle vasculature. This hypothesis implies that (a) V2R is expressed in the ECs lining the skeletal muscle vasculature, where the vasodilatory effect occurs and (b) that eNOS activation can occur in a V2R-dependent, cAMP-mediated manner.

(a) By quantitative RT-PCR, we found V2R to be expressed not only in human kidney, but also in lung, heart and skeletal muscle [57]. We have so far not been able to demonstrate V2R expression in skeletal muscle ECs. However, we have found V2R expression in HMVEC-L [32], and assume that this receptor is expressed in ECs in other sites. A more detailed tissue and cell type distribution study of V2R expression remains to be done.

(b) eNOS is known to be activated by stimuli such as acetylcholine, and bradykinin which act via an increase in $[Ca^{2+}]_i$ and binding of calmodulin to eNOS [58,59]. eNOS can also be activated by phosphatidyl inositol 3-kinase (PI3K)-dependent phosphorylation of residue Ser1177 by stimuli such as fluid shear stress, VEGF, and estradiol [60–62]. Several reports indicate that cAMP-raising agents induce eNOS activation via direct phosphorylation of Ser1177 [63–65]. We have found that forskolin and epinephrine induce NO production (as measured by autocrine cGMP production) and PI3K-independent eNOS activation (as detected by enzyme activity measurements and eNOS Ser1177 phosphorylation) [57]. We attempted to show V2R-mediated eNOS activation in HMVEC-L, but eNOS expression in these cells was too low for reliable detection. However, we were able to demonstrate that heterologous expression of V2R in HUVECs reconstitutes DDAVP-induced, cAMP-dependent eNOS activation [57]. Thus, both cellular and physiological data strongly suggest that DDAVP induce vasodilation by direct, V2R- and cAMP-mediated eNOS activation in ECs of resistance vessels.

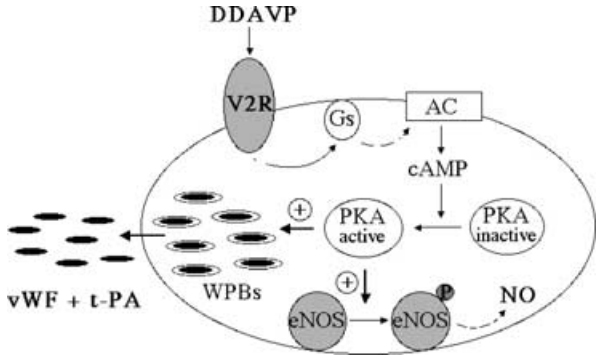

Fig. 1. Model for the mechanism of DDAVP-induced VWF and t-PA secretion, and of eNOS activation. DDAVP binds to endothelial V2R, leading to activation of a trimeric G-protein (Gs), activation of adenylyl cyclase (AC), production of cAMP, and activation of protein kinase A (PKA). Activation of PKA stimulates the exocytosis of WPBs, leading to the coordinate release of VWF and t-PA. It also induces eNOS activation, by phosphorylation (P) of residue Ser1177, leading to NO production.

Our current model for the effects of DDAVP on VWF, t-PA, and NO production are summarized in Fig. 1.

The effect of DDAVP on FVIII plasma levels

FVIII is the cofactor of activated coagulation FIX, responsible for the activation of FX of the intrinsic coagulation pathway, leading to the formation of a fibrin clot (reviewed in [66]). FVIII is also the factor that is missing or defective in hemophilia A.

The effect of DDAVP on circulating FVIII levels remains poorly understood. The plasma level of any substance results from the balance between production and removal. Thus, DDAVP could induce FVIII release from its producing cells. Alternately, FVIII could be protected from proteolytic degradation, e.g. by DDAVP-induced increase in plasma VWF.

DDAVP-induced FVIII production

The hypothesis that DDAVP induces cellular release of FVIII remains poorly documented. From the perspective of DDAVP physiology, the cell type producing FVIII should feature FVIII storage and a DDAVP-activated, V2R-mediated pathway of regulated secretion. One difficulty is the continuing uncertainty about the precise site of synthesis and release of FVIII in humans. It is generally accepted that FVIII is produced by the liver, although FVIII expression has also been demonstrated in several additional tissues, notably in spleen, kidney, and lymph nodes [67,68]. Within the liver, early studies have demonstrated FVIII expression in liver sinusoidal ECs by immunohistochemical techniques [69–71]. However, no FVIII mRNA could been detected in these cells in humans [67]. These observations might reflect surface binding or internalization of FVIII. However, in cellular terms, it is difficult to imagine a DDAVP-sensitive storage and release compartment that is replenished solely by FVIII internalization. Furthermore, induction of a rise in FVIII plasma levels by VWF administration to a VWD type 3 patient (with severe deficiency in both VWF and FVIII) did not allow for the reestablishment of a DDAVP releasable pool of FVIII [72]. Several studies have demonstrated expression of FVIII in hepatocytes [67,68], data strengthened by the observation that the FVIII promoter contains elements characteristic for hepatocyte-specific expression [73]. However, hepatocytes are thought not to display protein storage and regulated secretion [74]. Thus, hepatocytes may contribute to steady state levels of plasma FVIII, but not to acute DDAVP-induced increases in FVIII. In mouse, FVIII mRNA was recently detected in sinusoidal ECs, as well as in hepatocytes and Kupffer cells [75,76]. It might be interesting to reinvestigate FVIII mRNA expression in sorted human liver sinusoidal ECs and hepatocytes to clarify the question of FVIII expression in these cells.

One hypothesis is that FVIII is stored and released from WPBs. Rosenberg *et al*. have shown that heterologous expression of FVIII in ECs results in FVIII storage and release, together with VWF [77,78]. These findings demonstrate binding of FVIII to VWF even in intracellular compartments. However, hepatocytes do not express VWF. Likewise, sinusoidal ECs are generally thought not to express VWF, and do not contain any WPBs [79–81]. Contradictory results have been published concerning FVIII expression in other ECs. FVIII expression was reported in liver vein and arteries, as well as in placenta, lung and spleen ECs [71], while in other studies FVIII expression was not detected outside liver sinusoidal ECs [70].

DDAVP-induced FVIII increase: an indirect effect via VWF secretion?

FVIII plasma levels are predicted to a significant extent by plasma VWF levels in the general population [82]. Each VWF monomer binds one molecule of FVIII with high affinity, to form a tight, non-covalent complex that protects FVIII from proteolytic degradation [66]. Type 3 VWD patients have neither plasma VWF nor FVIII, showing the dependency of FVIII plasma levels on VWF expression. Thus, one simple hypothesis is that the effect of DDAVP on FVIII is indirect, via VWF secretion, making more FVIII binding sites available. If this hypothesis is correct, infusion of VWF should mimic the effect of DDAVP on FVIII levels. Indeed, infusion of VWF to VWD patients led to acute restoration of FVIII levels, albeit with a significantly slower time course than after DDAVP [72,83].

Further, the plasma VWF/FVIII molar ratio is approximately 50, suggesting a vast excess of available FVIII binding sites on VWF [84]. Since VWF is hardly a limiting factor for FVIII/VWF interaction, it is difficult to envisage how a DDVAP-induced rise in FVIII levels could be accounted for by VWF secretion. Again, the correlation between FVIII and VWF plasma levels (both in steady state and after DDAVP) could be explained by cosecretion, but a cell type that stores and secretes both proteins has not yet been identified.

DDAVP tachyphylaxis

When patients with VWD or mild hemophilia A, or normal control subjects are treated with DDAVP doses repeated over short time intervals (< 24 h), a decreased biological response is observed, a phenomenon called tachyphylaxis [85]. The magnitude of reduction is highly variable between patients. The cellular mechanisms underlying tachyphylaxis are unknown. V2 receptor desensitization, as occurs with other receptors after repeated stimuli, is one possibility. In normal individuals, a second infusion causes a reduced VWF and FVIII response, whereas the t-PA response is unaffected [86]. One explanation for that observation is that t-PA but not VWF stores are replenished before the next DDAVP infusion, due to the slower rate of endothelial VWF synthesis and cellular processing.

Other hemostatic mechanisms of DDAVP: also related to endothelial secretion?

The effects of DDAVP on FVIII and VWF levels are well established; are they sufficient to explain the hemostatic effect of the drug? The answer is likely negative, especially in

conditions such as uremia or platelet disorders, where VWF or FVIII levels are not decreased. Our model proposes that DDAVP induces VWF and t-PA release from WPB. It is important to remember that exocytosis of VWF from WPB occurs both into the vascular lumen and the subendothelium [87]. Oral mucosa biopsies taken after DDAVP administration demonstrate the presence of extracellular, cell-associated VWF, that may participate in platelet adhesion to the vascular endothelial cell surface [88,89]. Freshly released, cell-associated VWF is highly multimerized, and therefore more active in terms of platelet adhesion than circulating VWF [13].

DDAVP is expected to induce release of other WPB constituents, notably P-selectin, IL-8, CD-63, and endothelin-1 [90]. P-selectin is expressed in both ECs and megakaryocytes/platelets, in WPBs and α-granules, respectively [91–93]. P-selectin translocation to the endothelial cell surface promotes leukocyte rolling, an early step in leukocyte extravasation [94,95]. Similarly, P-selectin expression at the endothelial cell surface promotes platelets rolling through interaction with the P-selectin counterreceptors PSGL-1 and GP1b [96,97]. In mouse, a deficit in P-selectin has been shown to impair hemostasis [98], as well as the early inflammatory response [99,100]. DDAVP was shown to stimulate the exocytosis of P-selectin in cultured human ECs as well as in rat postcapillary venules [101]. Thus, DDAVP may induce P-selectin-mediated platelet and leukocyte adhesion on the endothelial cell surface.

Several reports have shown a direct effect of DDAVP and AVP on platelet activation [102–104]. However, the cellular mechanisms involved have not been defined. Further, V2R expression in platelets has not been demonstrated.

Although the mechanisms of DDAVP-induced endothelial activation are now well understood, the effect on FVIII and likely additional hemostatic mechanisms remain to be addressed.

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