Specificity in the Factor VIII Response to Vasopressin Infusion in Man

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Summary. Structural requirements of the systemic factor VIII response to intravenous vasopressin in man has been investigated using vasopressin analogues. With the analogues available the receptor specificity of this phenomenon could not be distinguished from those associated with the previously described plasminogen activator release or antidiuretic effects of this hormone. Further studies using 1-desamino-[8-D-arginine]vasopressin showed a dose-related release of both procoagulant and antigenic components of the factor VIII complex. The newly released factor VIII could not be distinguished from circulating factor VIII on the basis of molecular size, electrophoretic mobility or *in vitro* stability despite apparent differences in the duration of response of the procoagulant and antigenic components *in vivo*.

A recently introduced vasopressin analogue, 1-desamino-[8-D-arginine]vasopressin (dDAVP) has been shown to trigger a sustained release of factor VIII without the more unpleasant side effects which accompany the administration of vasopressin or adrenaline, and following its intravenous infusion into patients with mild haemophilia A or von Willebrand's disease sufficient biologically active factor VIII is released into the blood to permit modest surgical procedures without replacement therapy (Mannucci *et al*, 1977). dDAVP also has powerful antidiuretic properties and stimulates the release of the fibrinolytic proenzyme, plasminogen activator (Cash *et al*, 1974). The former has given cause for concern, producing significant water retention in a haemophiliac (Lowe *et al*, 1977); and the latter is a potentially undesirable side-effect in patients with pre-existing haemostatic inadequacy undergoing surgery.

The following communication describes studies in which a series of vasopressin analogues have been given intravenously to healthy volunteers in order to explore the possibility that an analogue may be made available which triggers a factor VIII response which is largely devoid of these other unwanted side-effects. In addition, preliminary experiments were performed to examine the nature of the factor VIII released in response to dDAVP.

MATERIALS AND METHODS

Materials. Synthetic peptides were obtained from various sources and reconstituted in sterile

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solution (Ferring, Malmo) prior to infusion, as described by Cash *et al* (1978). The source and structure of each peptide and the dose used are shown in Table I.

Infusions. Intravenous infusion of the different vasopressin analogues was carried out as described previously (Cash *et al*, 1978). Briefly, infusions were carried out between 09.00 and 11.00 hours. Subjects were rested supine for 30 min and isotonic saline was then infused over the next 15 min into a cubital fossa vein, by means of a constant infusion pump via a 19 gauge butterfly needle. Vasopressin (dissolved in 50 ml isotonic saline) was infused between the fifteenth and thirtieth minute through the same needle. Blood samples were taken at the indicated times from a similar needle in the contralateral arm and kept patent by a slow infusion of saline. The same procedure was used in studies of the factor VIII response to different dDAVP doses except that an additional blood sample was taken 4 h after the infusion. In this study a separate group of five male volunteers (26–40 years old) each received three different doses at intervals of at least 1 week.

Assays. After separation, plasma samples for the assay of factor VIII were frozen and stored at −40°C immediately. All samples from a single infusion were then thawed and assayed together, usually on the same day as the infusion, but never more than 7 d later.

Factor VIII procoagulant activity (VIII–AHF) was assayed by a modification of the activated partial thromboplastin time using severe haemophilic plasma as the substrate (Hardisty & Macpherson, 1962, as modified by Veltkamp, 1967). The assays were standardized against freeze-dried plasma of known factor VIII content, either the Fourth or Sixth British Standard (obtained from the National Institute for Biological Standards and Control, Holly Hill, Hampstead, London). Some samples were also assayed by the two-stage technique of Biggs *et al* (1955) using the same factor VIII standards.

Factor VIII-related antigen (VIII–AGN) was assayed by the Laurell method (1972) using 0.4% antiserum (Behringwerke A.G., Marburg, Germany) in 1% agarose (Indubiose, L'Industrie Biologique Française, Gennevilliers, France). The buffer used was tris-EDTA-borate, pH 8.6 (Aronsonn & Grönwall, 1957) diluted five-fold. Results were expressed as a percentage of the antigen level in the same freeze-dried plasma used to standardize the bioassay. Crossed immunoelectrophoresis was carried out by a similar modification of the original technique (Laurell, 1972). The following coagulation tests were also carried out: platelet count, plasma fibrinogen, partial thromboplastin time, serum fibrin–fibrinogen degradation products and ethanol gelation test.

Data are presented as mean ± standard error of the mean (SEM) and significance values were calculated using an unpaired *t*-test.

Gel filtration. 0.5 ml samples of fresh plasma were applied to a 0.9 × 30 cm column of Sepharose CL-4B (Pharmacia Fine Chemicals AB) equilibrated at room temperature with 0.135 M NaCl, 0.014 M trisodium citrate, 0.01 M epsilon aminocaproic acid, 0.003 M sodium azide, pH 7.3. The column was run at 15 ml/h, and 0.5 ml fractions collected into polystyrene tubes. Aliquots of each fraction were assayed for VIII–AHF and VII–AGN as soon as the run was completed. Protein in each fraction was estimated by a modified Lowry method (Eggstein & Kreutz, 1955) using human serum albumin (Koch-Light, Colnbrook, England), as a standard.

In vitro decay. Samples of plasma were incubated at 37°C in plastic tubes and assayed for VIII–AHF by the one-stage method after 0, 3 and 5 h against freshly thawed samples of pooled normal plasma. Results were expressed as a percentage of the initial value.

TABLE I. Sequence, dose and suppliers of the infused peptides and the parent hormones. All residues in the L-configuration unless specified as D. d = I-desamino. Me = Methyl.

Peptide	Sequence	Symbol	Dose (µg)	Source
Desmopressin	$\begin{array}{ccccccccc} 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 \end{array}$ d-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-D.Arg-Gly-NH$_2$ S————S	dDAVP	10	Ferring Pharmaceuticals A.B., Malmo
6-Monocarba desmopressin	d-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-D.Arg-Gly-NH$_2$ S————CH$_2$	dCDAVP	10	K. Jost, Inst. Org. Chem. Biochem. Acad. Sci., Prague
1-Desamino-[8-MeArg] vasopressin	d-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-N.MeArg-Gly-NH$_2$ S————S	dNMe-AVP	10	J. Hlavacek, Inst. Org. Chem. Biochem. Acad. Sci., Prague
N-fragment	NH$_2$-Cys-Tyr-Phe-OH	—	10	E. Kasafirek, Res. Inst. Pharm. Biochem., Prague.
C-fragment	NH$_2$-Pro-Arg-Gly-NH$_2$	—	10	L. Carlsson, Ferring A.B., Malmo
Arginine vasotocin	NH$_2$-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Arg-Gly-NH$_2$ S————S	AVT	10	D. Gillessen, Roche, Basle

440 *C. V. Prowse et al*

RESULTS

Table II summarizes the effects of infusion of the various peptides on the level of VIII-AHF. Only dDAVP and dCDAVP produced a significant rise. A similar effect was seen when VIII-AGN was assayed. The significant results are shown in Table III, other peptides having no effect. The absence of the terminal alpha-amino group, the introduction of a thioether rather than a disulphide bridge and the substitution of the D-stereoisomer at position 8 are designed to reduce the degradation of vasopressin by plasma enzymes (Cort *et al*, 1977). These alterations do not affect the ability of vasopressin to release factor VIII. On the other hand, a change in the orientation of the omega amino group at position 8, as in dNMeAVP, or substitution of hydrophobic leucine for polar arginine, as in oxytocin (Cash *et al*, 1974), results in loss of activity. Similarly the aromatic phenylalanine side-chain at position 3 is necessary since AVT,

TABLE II. VIII-AHF (international units/ml) response in venous blood following infusion of synthetic peptides (for symbols see Table I). Infusion was from just after the fifteenth to the thirtieth minute. Mean ± SEM from five subjects.

| | *Time (min)* | | | | | |
	0	*7½*	*15*	*30*	*45*	*60*
dDAVP	0.98±0.12	1.03±0.12	0.98±0.11	2.10±0.41*	1.66±0.23†	2.04±0.30*
dCDAVP	0.99±0.11	0·97±0.15	1.03±0.08	1.65±0.11*	2.08±0.16*	1.99±0.16*
dNMeAVP	1.00±0.07	1.08±0.09	1.04±0.11	1.06±0.10	1.02±0.10	1.07±0.10
N-fragment	0.81±0.09	0.81±0.08	0.77±0.08	0.82±0.06	0.83±0.07	0.90±0.10
C-fragment	0.91±0.06	0.99±0.07	0.97±0.08	1.08±0.09	1.00±0.09	1.00±0.10
AVT	0.92±0.07	0.78±0.06	0.85±0.06	0.88±0.04	0.92±0.01	0.96±0.03
Saline	0.98±0.07	1.03±0.07	0.92±0.08	0.95±0.06	0.99±0.09	0.97±0.09

* Indicates a significant ($P < 0.01$) increase over the mean pre-infusion level.
† Four observations only.

TABLE III. VIII-AGN (as % standard plasma) response to infusion of synthetic peptides. All details as for Table II. Only the response to active peptides are shown, all others producing no change in the level of antigen.

| | *Time (min)* | | | | | |
	0	*7½*	*15*	*30*	*45*	*60*
dDAVP	121±16	104±12	119±18	134±17	167±16	181±8*
dCDAVP	126±14	129±15	135±21	150±19	215±29*	214±20*
Saline	117±19	119±19	128±20	125±15	128±17	131±18

* Indicates a significant ($P < 0.01$) increase over the mean pre-infusion level.

with an isoleucine at this position, is also inactive. Neither of the terminal tripeptides had any activity.

In a more detailed study of the effects of dDAVP volunteers were infused with three different doses of this drug. Tables IV and V summarize the results of this experiment. With the

TABLE IV. Response of factor VIII complex in venous blood to infusion of various doses of dDAVP. Details as described in the text and Table II.

	Time (min)					
	0	*7½*	*15*	*30*	*60*	*240*
One-stage VIII-AHF (i.u./ml)						
5 μg	0.86±0.10	0.83±0.10	0.81±0.09	1.08±0.13	1.34±0.20*	1.27±0.17*
10 μg	1.12±0.14	1.12±0.18	1.12±0.14	1.77±0.25*	2.05±0.30*	1.75±0.27*
20 μg	0.91±0.05	0.86±0.08	0.88±0.06	1.55±0.13*	2.39±0.27*	2.20±0.22
Two-stage VIII-AHF (i.u./ml)						
5 μg	0.98±0.08	0.96±0.09	0.92±0.08	1.26±0.13	1.45±0.09*	1.37±0.14*
10 μg	1.13±0.09	1.13±0.09	1.10±0.12	1.62±0.09*	1.81±0.10*	1.71±0.14*
20 μg	1.16±0.09	1.17±0.07	1.08±0.07	2.15±0.19*	2.23±0.19*	2.03±0.17*
VIII-AGN (% standard plasma)						
5 μg	89±9	88±10	88±8	113±10	145±23*	157±20*
10 μg	109±8	103±10	103±7	143±10*	181±25*	197±34*
20 μg	108±7	101±10	102±9	172±13*	264±28*	280±28*

Mean ± SEM for five subjects.
* Indicates a significant (*P* < 0.01) increase over the mean pre-infusion level.

TABLE V. Pulse rate (per minute) and arterial blood pressure during infusion of different doses of dDAVP. Blood pressure as mean pressure calculated as (diastolic +0.33 pulse pressure) in Torr. Mean value ±sem for five subjects.

	Time (min)					
	0	*7½*	*15*	*21*	*30*	*60*
Pulse rate						
5 μg	72±5	72±6	70±7	79±7	82±5	73±10
10 μg	78±6	76±3	74±7	82±6	88±8	75±5
20 μg	70±4	71±4	69±4	101±11*	91±5*	79±4
Blood pressure						
5 μg	85±5	84±5	84±5	80±8	82±6	84±5
10 μg	92±4	92±4	88±5	84±5	81±5	82±4
20 μg	87±4	87±5	88±4	81±3	82±3	86±4

* Indicates significant increase over mean pre-infusion level (*P* < 0.01).

442   C. V. Prowse et al

highest dose (20 μg) the increase in pulse rate became significant ($P < 0.01$). This was accompanied by facial flusing and a slight feeling of nasal congestion or drowsiness, symptoms that were noted to minor degree with the lower doses.

As illustrated in Fig 1, both VIII-AHF (assayed by one- or two-stage techniques) and VIII-AGN show a definite dose–response relationship, although the release of VIII-AGN was more prolonged. In two infusion experiments more frequent samples were taken and it was confirmed that VIII-AHF rose to a maximum at about 30 min after infusion (Fig 2). VIII-AGN reached its highest level in the 4 h post-infusion sample (Table III and Fig 1).

In view of this discrepancy between VIII-AHF and VIII-AGN responses the molecular characteristics of the post-infusion VIII complex were investigated more fully. Fresh plasma samples from various times after infusion of 20 μg dDAVP were subjected to gel filtration. All of these runs showed that the peaks of activity of both VIII-AHF and VIII-AGN were coincident and at the void volume, suggesting that both components have large apparent molecular weights and may be chemically associated. Representative profiles are shown in Fig 3. Recovery of VIII-AHF from the column was quantitative, whereas the VIII-AGN yield was variable, and in some cases as low as 50%. No explanation can yet be given for this phenomenon.

Enzymic degradation of factor VIII may produce an apparent rise in VIII-AGN due to the increased electrophoretic mobility of the degradation products (Pasquini & Hershgold, 1973; McKee et al, 1975; Van Mourik et al, 1977), with concomitant loss of VIII-AHF activity. Such mobility changes may be detected by crossed immunoelectrophoresis. Fig 4 shows that no such increase in mobility occurred and confirms the integrity of the VIII-complex suggested by the gel filtration experiments.
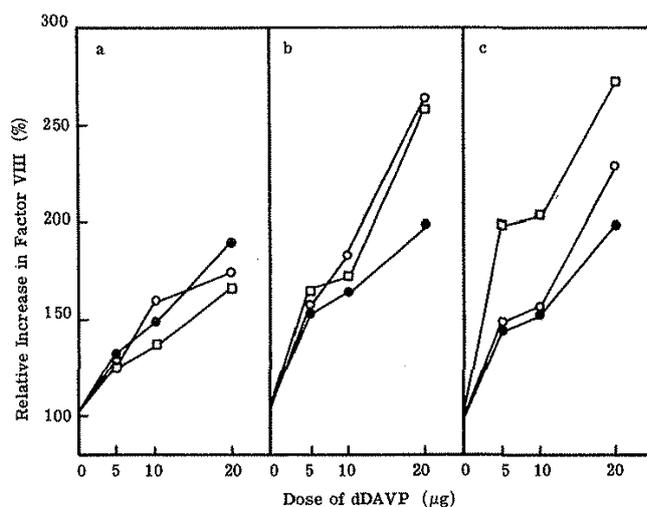

FIG 1. Responses of factor VIII blood levels assayed by one-stage (o) and two-stage (•) procoagulant assays, and of VIII-AGN (□), to varying doses of dDAVP. Separate curves are shown for the responses immediately after the end of infusion (a), 30 min (b) and 4 h later (c). Responses were expressed as % of the three resting values for each infusion.
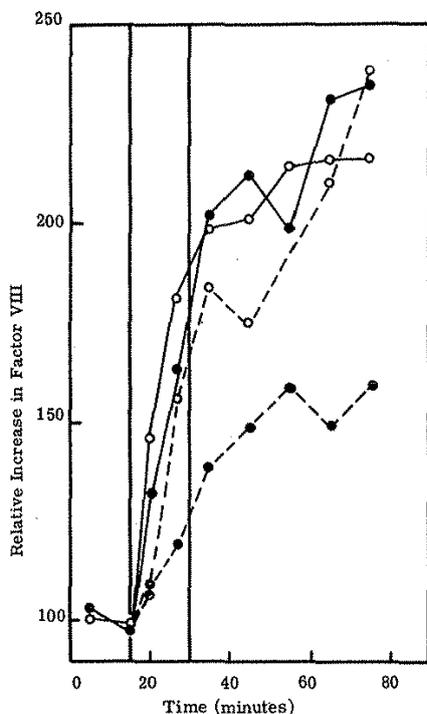
FIG 2. Responses of two subjects (o, •) to a 20 μg infusion of dDAVP. More frequent samples were taken than in the other experiments and the rises in VIII–AHF (solid line) and VIII–AGN (dashed line) are expressed as % of the mean resting level.
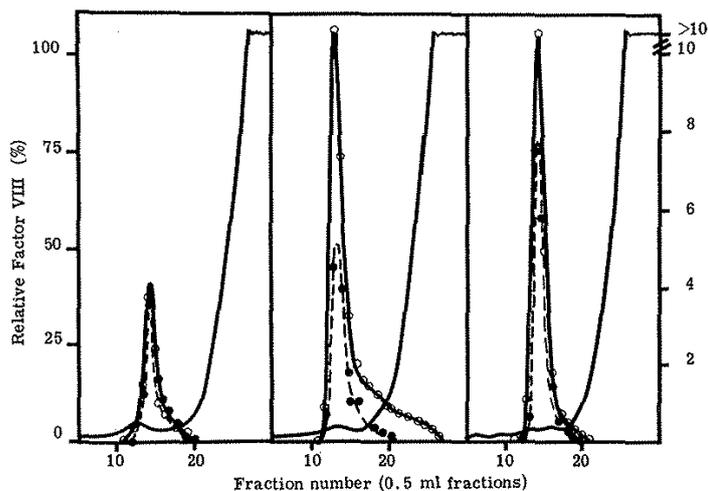

FIG 3. Gel filtration of fresh plasma samples on Sepharose 4B taken before (a), 30 min after (b), and 4 h after (c) the infusion of 20 μg of dDAVP. These are representative runs and were not taken from the same infusion. Protein concentration in mg/ml shown on right-hand scale (—), VIII–AHF (o) and VIII–AGN (•) were assayed in each fraction.
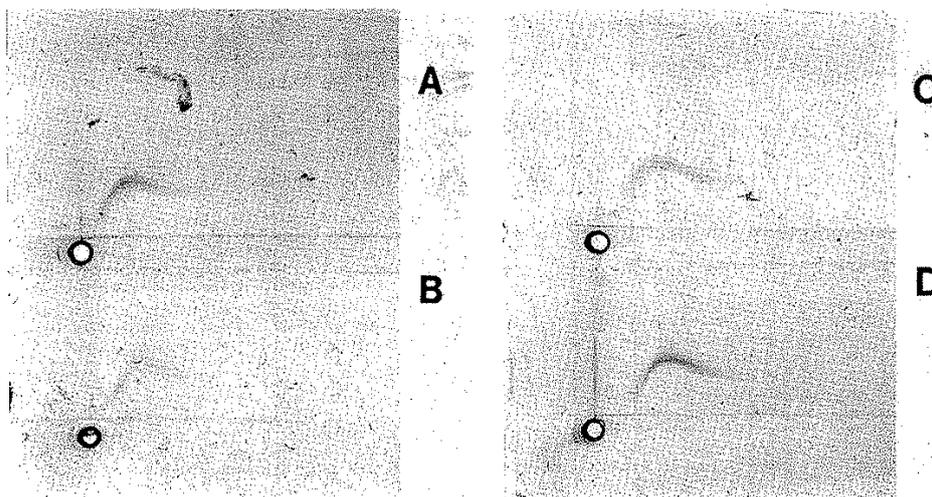
C. V. Prowse et al

FIG 4. Crossed immunoelectrophoresis of plasma samples from a 20 μg infusion of dDAVP taken before (A), 30 min (B), 1 h (C), and 4 h (D) after infusion, against antiserum to factor VIII related antigen.

Weiss *et al* (1977) have recently suggested that the association of VIII-AHF and VIII-AGN confers stability on the AHF component. If this is so, dissociation of the two components might result in greater thermolability of the VIII-AHF. Samples of plasma from 30 min post-infusion samples were tested for this possibility. Fig 5 shows that no significant difference was found between the rate of decay in such samples and those taken from untreated subjects.
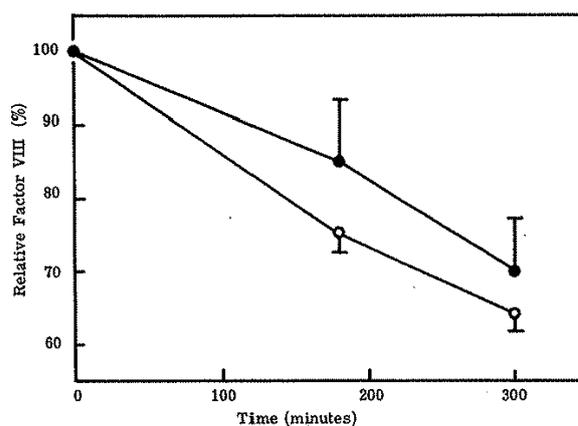

FIG 5. The decay of VIII-AHF in plasma of normal subjects (o, *n*=10) and in samples taken 30 min after a 20 μg infusion of dDAVP (●, *n*=3) during incubation at 37°C. Results are shown as the mean decrease relative to the initial level ± SEM. No significant difference is apparent.

DISCUSSION

It is apparent from the results of infusion of the various vasopressin analogues described above, together with those from the earlier study of Cash *et al* (1974), that the receptor for factor VIII release requires the integrity of the whole of the cyclic nonapeptide structure and that there is a requirement for the aromatic residue at position 3 and an omega amino group at position 8. On the other hand, the receptor is not sensitive to the loss of the N-terminal alpha-amino group, the substitution of a thioether for the disulphide bridge in the ring or to the stereoisomer of the position 8 residue. This profile is very similar to that for the plasminogen activator releasing (and antidiuretic) receptor for vasopressin but differs from that for smooth muscle pressor activity, which requires the L-stereoisomer at position 8 (Cort *et al*, 1975). The only difference we found was with arginine-vasotocin, which has a slight plasminogen activator releasing activity (Cash *et al*, 1978) but induces no significant increase in factor VIII. This may well be a dose-related effect since preliminary studies indicate that the different amounts of dDAVP used in this study produce a near maximal release of plasminogen activator, whereas factor VIII release exhibits a dose–response curve of lesser slope. Taken together with earlier work (Cash *et al*, 1978; Cort *et al*, 1977) it is apparent that the analogues used do not distinguish between the receptors for factor VIII and plasminogen activator release or antidiuretic activity, while the receptor for the pressor activity of vasopressin does have a distinct specificity.

Mannucci *et al* (1975, 1977) have shown that both VIII-AHF and VIII-AGN increase after infusion of vasopressin. In the parallel situation of adrenalin infusion there is disagreement on whether these two activities rise proportionately (Bennett & Ratnoff, 1972; Prentice *et al*, 1972; Ingram *et al*, 1977). This study has shown that both VIII-AHF and VIII-AGN rise in a dose dependant manner following dDAVP infusion. Immediately following infusion the rise in these activities was comparable. There was a slight delay between VIII-AHF and VIII-AGN increases, confirming the results of Mannucci *et al* (1975). The results of Stibbe (1977) using exercise as the stimulus for release, could be similarly interpreted. The rise in VIII-AGN was more prolonged and 4 h after infusion this was higher than that of VIII-AHF. This could have resulted from enzymic degradation of VIII, which would also explain the differences between the one- and two-stage assays of VIII-AHF in the 30 min post-infusion samples (Fig 1). However, a number of facts make this unlikely. The apparent molecular sizes of VIII-AHF and VIII-AGN, as determined by gel filtration elution volumes, were the same regardless of the time after infusion. Similarly, electrophoretic mobilities and *in vitro* stability of post-infusion samples did not change with time. In addition, VIII-AHF was still elevated 4 h after the infusion, suggesting that the newly released VIII-AHF was relatively stable *in vivo*.

Further evidence of the physiological nature of freshly released factor VIII is provided by its known clinical efficacy and its comparatively normal survival *in vivo* following dDAVP infusion of haemophiliacs (Mannucci *et al*, 1977). Adrenalin infusion of normal subjects similarly produces VIII-AHF with a normal half-life (Ingram *et al*, 1977) whereas in von Willebrand's disease this treatment produces VIII-AHF with a reduced survival time (Rickles *et al*, 1976). In view of the observation of Weiss *et al* (1977) that VIII-AGN stabilizes VIII-AHF *in vitro*, the effect described by Rickles *et al* (1976) could be related to the relatively minor increase in VIII-AGN induced in these patients by adrenalin infusion.

The differences in rate of release of VIII-AHF and VIII-AGN are unlikely to be due to

446   *C. V. Prowse et al*

depletion of the storage pool of one component since they are observed even with low doses of peptide agonist. Rickles *et al* (1976) suggested that apparent differences may occur if newly released factor VIII complex has a different stoichiometry from the circulating complex. This would not account for the present situation since early post-infusion samples contained comparable levels of VIII-AHF and VIII-AGN, differences only becoming apparent in later samples.

One possibility is that vasopressin induces a release of only one component of the complex, the other entering the circulation as a secondary event. This is supported to some extent by the findings of Jaffe *et al* (1973) that cultured human endothelial cells can synthesize VIII-AGN but not VIII-AHF.

In conclusion, it is apparent that vasopressin infusion results in a dramatic increase in circulating factor VIII. The use of vasopressin analogues allows this to occur without the unpleasant side effects of the parent molecule, and with prolonged response duration. At present it is not possible to separate the factor VIII response from plasminogen activator release or the antidiuretic action of the hormone, although preliminary results with vasotocin and dDAVP suggest that there are potency differences in the relative dose–response relations which could be exploited in further synthetic variations of the molecule. Although some assays suggest that the factor VIII released by vasopressin is in some way different from that which normally circulates, stability tests, gel filtration and electrophoresis do not reveal this. Furthermore, the released complex is known to be haemostatically effective.

REFERENCES

ARONSONN, T. & GRÖNWALL, A. (1957) Improved separation of serum proteins in paper electrophoresis—a new electrophoresis buffer. *Scandinavian Journal of Clinical and Laboratory Investigation*, 9, 338–341.

BENNETT, B. & RATNOFF, O.D. (1972) Changes in antihemophilic factor (AHF, factor VIII) procoagulant activity and AHF-like antigen in normal pregnancy, and following exercise and pneumoencephalography. *Journal of Laboratory and Clinical Medicine*, 80, 256–263.

BIGGS, R., EVELING, J. & RICHARDS, G. (1955) The assay of antihaemophilic-globulin activity. *British Journal of Haematology*, 1, 20–34.

CASH, J.D., GADER, A.M.A. & DA COSTA, J. (1974) Release of plasminogen activator and factor VIII in response to LVP, AVP, dDAVP, angiotensin II and oxytocin in man. (Abstract). *British Journal of Haematology*, 27, 363–364.

CASH, J.D., GADER, A.M.A., MULDER, J.L. & CORT, J.H. (1978) Structure–activity relations of the fibrinolytic response to vasopressins in man. *Clinical Science and Molecular Medicine*, 54, 403–409.

CORT, J.H., ALBRECHT, I., NOVÁKOVÁ, J., MULDER J.L. & JOŠT, K. (1975) Regional and systemic haemodynamic effects of some vasopressins; structural features of the hormone which prolongs activity. *European Journal of Clinical Investigation*, 5, 165–175.

CORT, J.H., CASH, J.D., JOST, K., SCHWARTZ, I.L. & MULDER, J.L. (1977) The cyclic neurohypophysial peptides: from structure to clinical use. *Molecular Endocrinology* (ed. MacIntyre & Szelke), p. 337. Elsevier, Amsterdam.

EGGSTEIN, M. & KREUTZ, F.H. (1955) Vergleichende Untersuchungen zur quantitativen Eiweissbestimmung im liquor und eiweissarmen Lösungen. *Klinische Wochenschrift*, 33, 879–884.

HARDISTY, R.M. & MACPHERSON, J.D. (1962) A one-

stage factor VIII assay and its use on venous and capillary plasma. *Thrombosis et Diathesis Haemorrhagica,* **7,** 215–229.

INGRAM, G.I.C., VAUGHAN JONES, R., HERSHGOLD, E.J., DENSON, K.W.E. & PERKINS, J.R. (1977) Factor-VIII activity and antigen, platelet count and biochemical changes after adrenoceptor stimulation. *British Journal of Haematology,* **35,** 81–100.

JAFFE, E.A., HOYER, L.W. & NACHMAN, R.L. (1973) Synthesis of antihemophilic factor antigen by cultured human endothelial cells. *Journal of Clinical Investigation,* **52,** 2757–2764.

LAURELL, C.-B. (1972) Electroimmuno assay. *Scandinavian Journal of Clinical and Laboratory Investigation,* **29,** Supplement 124, 21.

LOWE, G., PETTIGREW, A., MIDDLETON, S. FORBES, C.D. & PRENTICE, C.R.M. (1977) D.D.A.V.P. in haemophilia. (Letter). *Lancet,* **ii,** 614–615.

MANNUCCI P.M., ÅBERG, M., NILSSON, I.M. & ROBERTSON, B. (1975) Mechanism of plasminogen activator release and factor VIII increase after vasoactive drugs. *British Journal of Haematology,* **30,** 81–93.

MANNUCCI, P.M., RUGGERI, Z.M., PARETI, F.I. & CAPITANIO, A. (1977) 1-Deamino-8-D-arginine vasopressin: a new pharmacological approach to the management of haemophilia and von Willebrands disease. *Lancet,* **i,** 869.

McKEE, P.A., ANDERSON, J.C. & SWITZER, M.E.

(1975) Molecular structural studies of human factor VIII. *Annals of the New York Academy of Sciences,* **240,** 8–33.

PASQUINI, R. & HERSHGOLD, E.J. (1973) Effects of plasmin on human VIII (AHF). *Blood,* **41,** 105–111.

PRENTICE, C.R.M., FORBES, C.D. & SMITH, S.M. (1972) Rise of factor VIII after exercise and adrenaline infusion measured by immunological and biological techniques. *Thrombosis Research,* **1,** 493–506.

RICKLES, F.R., HOYER, L.W., RICK, M.E. & AHR, D.J. (1976) The effects of epinephrine infusion in patients with von Willebrand's disease. *Journal of Clinical Investigation,* **57,** 1618–1625.

STIBBE, J. (1977) Effect of exercise on factor VIII complex: proportional increase of ristocetin cofactor and factor VIII-AGN but disproportional increase of factor VIII-AHF. *Thrombosis Research,* **10,** 163–168.

VAN MOURIK, J.A., HELLINGS, J.A. & HOORWEG, E.M. (1977) Microheterogeneity of human Factor VIII. (Abstract). *Thrombosis and Haemostasis,* **38,** 10.

VELTKAMP, J.J. (1967) Detection of carrier states in hereditary coagulation disorders. M.D. thesis, University of Leiden, The Netherlands. Schatteur-Verlag, Stuttgart.

WEISS, H.J., SUSSMAN, I. & HOYER, L. (1977) Stabilisation of factor VIII in plasma by the von Willebrand Factor. *Journal of Clinical Investigation,* **60,** 390–404.