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Antihaemophilic effect of vasopressin, deamino-(D-arginine^8)-vasopressin and adrenaline in sheep: proposal for an *in vivo* assay system

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Female sheep were used to assay antihaemophilic (factor VIII enhancing) activity of arginine vasopressin, deamino-(D-arginine^8)-vasopressin (DDAVP) and adrenaline. The time course of the response was biphasic, two surges of factor VIII being observed. DDAVP was found to be the most potent of the substances investigated. Its optimal dose was 1 $\mu g\,kg^{-1}$ body wt (i.v.). It is suggested that a similar procedure can be employed to search for new peptides with antihaemophilic action.

Introduction Several vasoactive substances of various generic groups, catecholamines (Ingram, 1961), vasopressins and nicotinic acid (Mannucci *et al.*, 1975; Prowse *et al.*, 1979) elicit an increase of blood clotting factor VIII: C (F-VIII) in man. In healthy individuals, symptomatic haemophilia A patients, carriers and patients with certain types of von Willebrand's disease, this 'antihaemophilic' action is particularly pronounced following administration of deamino(D-arginine^8)-vasopressin (DDAVP), a peptide with only a minute vasopressor potency (Mannucci *et al.*, 1975; 1977). The mechanism of action remains unexplained: the lack of correlation with vasoactivity argues against the 'squeezing' of F-VIII-producing endothelial cells. Furthermore, the absence of DDAVP-induced F-VIII release from human umbilical vein endothelial cells in culture (Moffat *et al.*, 1984) disproves any direct effect on the putative F-VIII producing cells in general. In this situation, the design of new peptides with even stronger antihaemophilic potency and/or a shift in the activity spectrum toward this potency is fraught with difficulties, made even greater by the lack of a reliable and standard *in vivo* bioassay. This short communication describes our assay results in sheep, a suitable animal model for screening purposes.

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Methods Female White Alpine sheep were used in the experiments. Substances were administered intravenously (bolus injection) into the jugular vein from which blood samples were also withdrawn by repeated venepuncture. Citrate plasma was used to estimate the plasma F-VIII level, employing a commercial F-VIII one stage assay kit (Merz + Dade, Düdingen, Switzerland). Relative F-VIII plasma levels were obtained by comparing the clotting times of the diluted plasma samples with those of the dilution curve obtained for the standard sheep plasma and/or the animal plasma collected before injection. The 'substrate' method (Rosén, 1984) yields very similar estimates, as found by comparison in a series of parallel assays.

Since not all animals (similar to man) display a F-VIII response to DDAVP, a group of 30 ewes was used for a preliminary search for 'reactants'. Eighteen animals responding to DDAVP, 1 $\mu g\,kg^{-1}$ body wt, with an F-VIII increase after 5 and 20 min were selected for experiments. Animals were then treated with various doses (in random order) of the assayed substances at 6 day intervals (replenishment of F-VIII pools); 7 to 10 blood samples were withdrawn within 120 min. Adrenaline was used in a dose-range 3 to 70 $nmol\,kg^{-1}$ body wt, arginine vasopressin (AVP) and DDAVP 0.03 to 8 $nmol\,kg^{-1}$. Assays of F-VIII were carried out within 45 min after the blood withdrawal. The area under the F-VIII curve between 0 and 120 min, related to the corresponding basal level, was taken as a response descriptor ('integrated response').

DDAVP and AVP were products of Ferring AB, Malmö, Sweden. Adrenaline hydrochloride was used at a concentration of 2 $mg\,ml^{-1}$ (isotonic saline).

Results and discussion In contrast to similar profiles in man (Mannucci *et al.*, 1975), the time course of F-VIII after DDAVP and AVP administration was biphasic. The first surge of F-VIII occurred within approximately 20 min (peak value), and the rebound phase lasted another 10 to 20 min. A second

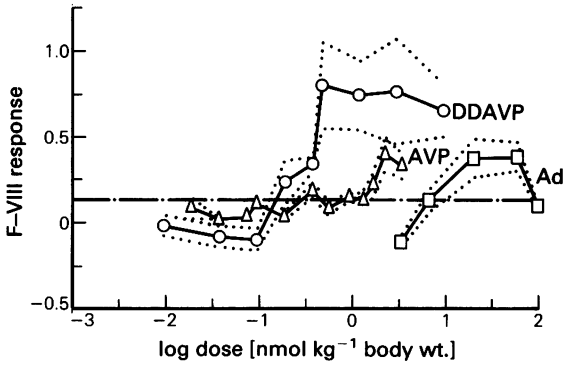

Figure 1 Dose-response curves for deamino- (D-arginine[8])-vasopressin (DDAVP, ○), arginine vasopressin (AVP, △) and adrenaline (Ad, □). For definition of response, see text. Dotted lines: standard errors estimated in experiments on various animals (3 to 15). Hatched line: detection limit estimated from blind experiments with physiological saline injection (mean ± s.e. mean of the 'blind' response, 8 animals). Response was expressed as an integral of the F-VIII time-course during an interval from zero to 120 min in relation to the corresponding integral of the basal level.

peak appeared within 1 to 1.5 h after the injection. The profile did not change after pretreatment with inhibitors of fibrinolysis (tranexamic acid, Trasylol) or by sedatives (Rompun, Bayer), thus proving that the pattern of this response was influenced neither by an initial treatment stress (first peak) nor by the DDAVP- or AVP-induced plasminogen activation (rebound phase after the first peak). The rebound phase was not explainable simply by a subsequent elimination of F-VIII: the half-life of F-VIII is presumably much longer.

Dose-response curves (Figure 1) of all three substances tested here displayed maxima at approx. 0.9, 3.7 and 44 nmol kg^{-1} body wt for DDAVP, AVP and adrenaline, respectively. The maximum response to DDAVP was roughly twice as great as that to AVP and adrenaline.

About 60% of all animals tested by us (about 250) responded positively to DDAVP and could therefore be used in the experiments. Clinical experience suggests a very similar percentage for man. The integrated response (see Methods) to 1 $\mu g\,kg^{-1}$ body wt DDAVP in the group investigated displayed a logarithmic-normal frequency distribution; this might in the future be employed for exclusion of 'low' and 'high' reactants by suitable statistical criteria and contribute to the standardization of the assay. The reactants did not show any regular F-VIII change following a sham treatment, such as injection of saline, injection of an inactive substance (angiotensin II) or a subthreshold dose of an active substance, or blood withdrawal in regular intervals without any injection.

Three further effects of potential interest but not yet analysed in detail, should be briefly mentioned. First, DDAVP at a dose of 1 $\mu g\,kg^{-1}$ elicited a clear-cut release of ACTH which was roughly 50% of that of AVP. The time course of ACTH release parallels that of the F-VIII response. Second, we have also observed an increase of plasma renin activity after DDAVP treatment, as found by Williams *et al.* (1986) in man. And third, all three substances assayed caused a rapid decrease of haematocrit up to 75% of the norm. The total plasma protein concentration decreased concomitantly by about 4 to 7%. The status was usually normalized after 2 h. Direct renal effects alone seem to be only partially responsible for this phenomenon.

Clinical interest in substances improving the status of haemophilic patients is motivated by high costs and high therapeutic demands for F-VIII substitution therapy, and by the acute danger of viral infectious diseases such as AIDS and hepatitis. However, DDAVP is not an ideal drug for these purposes, owing to several side effects described in the literature (cf. Sutor, 1980; 1981). The finding of more suitable peptides depends a great deal upon screening of a large group of selected substances. Although still not optimal, the bioassay on sheep yields basic antihaemophilic characteristics for the assayed substances, enables their comparison, and can therefore meet the requirements of the drug design. With the exception of the trained beagle dog (Vilhardt, *et al.*, 1987), the sheep is the only animal model available at present fulfilling these aims; small laboratory animals are quite unsuitable for these purposes.

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