Production of porcine antibodies with high specificity to [8-D-arginine] deamino-vasopressin (dDAVP)

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Summary. The development of a specific radioimmunoassay for [8-D-arginine] deamino-vasopressin (dDAVP) is described.

The production of antisera specific to neurohypophysial hormones[2,3] enabled a small quantity of these hormones to be detected in body fluids. In this report, the production of antiserum to [8-D-arginine]vasopressin (DAVP) is described in order to develop a radioimmunoassay for dDAVP; the specificity of this antiserum is studied.

Materials and methods. The following peptides were used in our study: [8-L-arginine]vasopressin (AVP), [8-lysine]-vasopressin (LVP), oxytocin (OT), [8-L-arginine]deamino-vasopressin (dAVP)[4], [8-D-arginine]deamino-vasopressin (dDAVP)[5] and [8-D-arginine]vasopressin (DAVP)[6]. All but AVP were synthetized in our Institute's Department of Organic Synthesis. AVP was isolated from natural material according to Prusík et al.[7]. DAVP was conjugated to rabbit immunoglobulin (rIgG) by the carbodiimide method[8,9]. The conjugate containing 3–4 moles DAVP per mole rIgG was emulsified with Al-Span-Oil adjuvant[10]. 2 pigs were immunized with this antigen according to Franěk and Šimek[10]. The labelling of synthetic dDAVP was performed according to Vaněčková et al.[9]; the labelled hormone was purified on columns of Dowex 1 × 8 and Sephadex G-25, its purity being checked by paper electrophoresis[11]. The preparation showed 1 sharp peak on the electrophoresis and its calculated specific radioactivity was 1000 Ci/mmole. The antiserum titre was determined by incubation of serial dilutions of the antiserum with labelled hormone (5000–10,000 cpm) in 0.1 M Tris-HCl buffer pH 7.8 containing 0.2% bovine serum albumin.

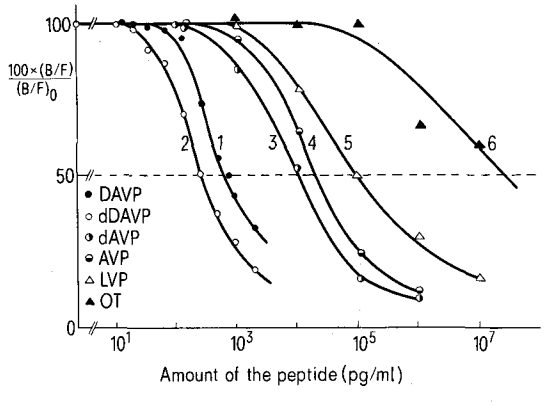
For the cross-reactivity studies, 100 µl of [^{125}I]-dDAVP (5000–10,000 cpm), 100 µl of unlabelled hormone (1–10^7 pg/ml of dDAVP, dAVP, DAVP, AVP, LVP and OT) and 400 µl of the antibodies at a dilution 1:2000 were incubated at 4 °C for 24 h. For the separation of free and bound hormone, dextran coated charcoal was used[9]. Aliquots of supernatant were counted. All these experiments were performed under such conditions that the maximal binding of labelled hormone to the antibodies in the absence of unlabelled hormone was 50 ± 3%, i.e. the bound to free ratio $(B/F)_0$ was 1 ± 0.1. The value $(B/F)_0$ was taken as 100% and all B/F values obtained for various concentrations of vasopressin analogues were related to this value. The values (B/F). $100/(B/F)_0$ were plotted against the log of the analogue concentration in the reaction. The concentration of the analogue resulting in 50% inhibition of binding was determined graphically.

Results and discussion. Our work aimed at obtaining antibodies to dDAVP. In this respect, the choice of the antigen for immunization was of crucial importance. We decided to use the conjugate of [8-D-arginine]vasopressin with rabbit immunoglobulin in the main for the following reason: it followed from our previous results on the specificity of porcine antibodies to AVP[9] that the immunoreactivity of the deamino analogue was at least as high as that of the amino one. From 2 immunized pigs we obtained only 1 antiserum, because 1 pig died before

Cross-reactivity of vasopressin analogues

Analogue	Percent cross-reaction at 50% displacement
dDAVP	125
DAVP	100
AVP	2
dAVP	5
LVP	0.1
OT	0.003

The values presented mean [the amount of DAVP required to displace 50% of labelled hormone/amount of analogue required to displace 50% of labelled hormone] × 100; for DAVP the concentration resulting in 50% displacement is 450 pg/ml.



Cross-reactivity of vasopressin analogues with the antibody to [8-D-arginine] vasopressin. Increasing amounts of different peptides are added to the incubation medium containing a constant amount of antibody and [^{125}I]-dDAVP. For details see 'materials and methods'.

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the end of the experiment. The titre of this antiserum determined radioimmunochemically was 1:2000. A radioimmunoassay for dDAVP was developed which permitted routine detection of 30 pg dDAVP/ml.

A study was made of the capacities of various analogues of AVP to inhibit the binding of [^{125}I]-dDAVP to the antibodies. The figure shows the dependence of the binding of labelled hormone on the unlabelled peptide concentration on logarithmic scale. The binding affinity of each substance to the antiserum relative to the binding affinity of DAVP is expressed as a percentage, i.e. 100 × [the amount of DAVP required to displace 50% of labelled hormone/amount of analogue required to displace 50% of labelled hormone], the values being presented in the table.

It is evident from the table that deamination did not reduce the affinity of the analogue to the antibodies. On the other hand, immunoreactivity of the natural hormones AVP, LVP and OT was lower. It can be seen from these data that the strong basicity and specific steric conformation of the amino acid in position 8 of the peptidic chain are important factors in the interaction with the antibodies. The specificity of porcine antibodies to DAVP shows the same regularity as the specificity of porcine antibodies to AVP[9]. We may conclude that, even though the sensitivity of the developed radioimmunoassay for dDAVP is 10–50 times lower than the sensitivity of recently developed RIAs for AVP[12–14], it can be useful for distribution, binding and metabolic studies of the dDAVP. The antibodies are specific enough not to interfere with the content of AVP, AVT and OT in biological material.

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Endocrine significance of critical periods during insect development: Analysis of ligation experiments with *Pieris brassicae* last instar larvae[1]

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Summary. Ecdysone haemolymph levels have been analyzed in neck- or thorax-ligated *Pieris* larvae in order to explain the physiological significance of critical periods. It appeared that head critical period corresponds to an incomplete activation of prothoracic glands, while the thoracic critical period is related to the secretion of a minimal amount of ecdysone necessary for moulting. During *Pieris'* last larval instar, there is no evidence for any noticeable synthesis of ecdysone in isolated abdomens.

Ligation experiments have been widely used with Lepidopteran larvae, due to particularly favourable morphological features. They led to the 'classical scheme' of moulting control by 2 hormones, a brain neurosecretion or prothoracicotropic hormone (PTTH) and a prothoracic gland (PG) steroid hormone, ecdysone. Ligatures define 2 critical periods, a head critical period related to PG activation by PTTH, and a prothoracic critical period corresponding to ecdysone synthesis and release by PG. While many experiments have confirmed the validity of this scheme, firstly established with *Bombyx mori*[3], some more recent observations invite caution, essentially due to the presence of noticeable PTTH activity in *Bombyx* abdomens[4], and the possibility of ecdysone synthesis outside PG in both sexes of several insect species[5–8]. Moreover, the position of critical periods appears to vary much more than expected, as for instance a critical period may take place in the foregoing stage[9]. It is therefore essential to get more direct information about the significance of critical periods, that is to determine the actual hormonal titers in ligated animals.

Materials and methods. We used last instar larvae of *Pieris brassicae*, where ecdysone levels and critical periods had previously been determined[10,11]. In the present work, animals carefully staged were ligated at various ages before and after the respective critical periods, and their haemolymph collected at regular intervals after ligations. The time of pupal ecdysis, when it occurred, was also recorded. Due to the small number of animals used for each haemolymph sample, ecdysone levels were determined using a radioimmunoassay (RIA) procedure[12].

Results. 1. Cephalic critical period. The diagram of figure 1A reminds the main events which occur during the last larval instar. The head critical period takes place during the feeding period. It corresponds to animals which have reached the $^2/_3$ of their maximum weight and coincides with the end of obligatory feeding period. If the larvae are ligated after the critical period, about 50% them normally ecdyse and transform themselves into headless pupae. The latter can survive for several months as long as they are maintained in moist conditions (the pupal cuticle is thinner than in controls) and they never give any sign

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