No changes were noted in cardiac output, heart rate, left ventricular stroke work or total peripheral resistance. A gradual decrease in mean systemic arterial pressure during the experiment was seen in all three groups. This is not uncommon in anesthetized swine and may explain the decreases in cerebral blood flow seen in the control group. There were no significant differences in mean arterial pressure among the three groups at any time period.

These results demonstrate that intravenous ME produces dose related increases in regional cerebral blood flow. Furthermore, these increases do not appear to correlate with changes in systemic hemodynamic parameters reported by others[13,14]. The present study does not address the question of whether the increases in blood flow seen are the result of a direct affect on cerebral vasculature or a physiologic response to a primary affect on neural tissue. It is of interest, however, that the areas most responsive to ME infusion also contain the greatest concentration of potential ME receptors.

Also unanswered is the question of whether this is a physiologic or pharmacologic response. If indeed, the enkephalinergic system plays a physiologic role in the regulation of intracerebral blood flow, failure of this system may be a component of diseases characterized by cerebral hypoperfusion such as senile dementia.

Additionally, although the question as to whether this effect in miniature swine is through endogenous enkephalinergic receptors is not resolved, future studies with opiate antagonists will help decide this point. This information, coupled with our results, may provide a rationale to stimulate research concerned with the potential therapeutic use of peptidyl opiates (analogs) as a treatment modality in conditions of reduced cerebral blood flow.

Acknowledgment. We wish to acknowledge the technical and secretarial assistance of Robert Ochoa and Sherri Wilke. This research was supported by a grant awarded to Kenneth Blum from Southwest Research Institute of San Antonio, Texas.

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0014-4754/85/070932-02$1.50 + 0.20/0

Use of three specific radioimmunoassays in measuring neurohypophysial hormone content and plasma concentrations of vasopressin, oxytocin and DDAVP in rats after prolonged infusion of DDAVP

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Summary. Specific radioimmunoassays (RIA) were employed for measuring plasma and neurohypophysial concentrations of oxytocin (OT) and vasopressin (AVP) after administration of 1-deamino-8-D-Arg-vasopressin (DDAVP). DDAVP concentrations were measured by a newly-developed specific RIA. Through the use of minipumps, DDAVP was infused i.p. over a period of 3 days in normally hydrated rats. Despite decreased urine production and increased urine osmolality no changes could be observed in neurohypophysial and plasma hormone concentrations.

Key words. Radioimmunoassay; oxytocin; vasopressin; neurohypophysis.

The use of the long-acting arginine vasopressin analogue 1-deamino-8-D-arginine vasopressin (DDAVP) in the treatment of central diabetes insipidus is widespread[1,2]. This hormone analogue also promotes the release of Factor VIII[3], making it useful for the treatment of mild hemophilia. Furthermore, DDAVP has been suggested for therapy of enuresis nocturna[4].

The purpose of this study was to investigate whether sustained antidiuresis obtained through long-term infusion of DDAVP would alter the neurohypophysial content of vasopressin (AVP) and oxytocin (OT) or plasma levels of these hormones in the rat.

Materials and methods. Peptide standards. DDAVP, AVP and OT were synthesized by Ferring, Malmö. The purity of these peptides was assessed by high performance liquid chromatography (HPLC) and was found to be greater than 95%.

Animal studies. Male Sprague-Dawley rats, weighing 180–220 g were placed under light Barbital® anesthesia. Osmotic minipumps (Alza®, model 2001) filled with a solution of DDAVP in bacteriostatic 0.9% NaCl were placed in the peritoneal cavity through a small upper abdominal incision. In preliminary experiments an infusion rate of 1 nmole/kg/day was found to give the desired plasma concentrations of DDAVP. The animals were placed in metabolic cages 3 days before surgery to acclimatize. Controls given 0.9% NaCl only, and experimental animals, were divided into groups of 10. The animal room was lighted from 06.00 to 18.00 h. 24-h urine collections were made for determination of urine volume (U_{vol}) and urine osmolality (U_{osm}). After 3 days the rats were decapitated and blood was collected in heparinized plastic tubes. After centrifugation at +4 °C for 15 min at 800 g_{av}, plasma was aspirated and stored at −70 °C until extraction. The dissected neurohypophyses were placed in an ice bath in 2 ml 0.1 M HCl, homogenized in a Tenbroeck glass homogenizer and centrifuged at 1600 g_{av} for 10 min. The supernatants were stored frozen at −70 °C until assayed.

Preparation of iodinated peptides. All peptides were iodinated with the iodogen method[5]. A polypropylene tube was coated with a thin layer of Iodogen (Pierce Co, USA). 5 nmoles peptide

dissolved in 0.05 M phosphate buffer, pH 7.5, were incubated together with 0.5 mCi $Na^{125}I$ in the iodogen tube. Incubation was continued for 30 min under constant vortexing. The incubate was then transferred to a 0.9 × 25 cm Sephadex G-25 (Fine) column (Pharmacia, Sweden), and equilibrated with 0.2 M acetic acid containing 0.1% human serum albumin. The peak fractions from this column were rechromatographed under identical conditions. This procedure produced high quality tracers with specific activities better than 1000 Ci/mmole.

Radioimmunoassays. A phosphate-buffer containing 0.15 M NaCl, 0.1% human serum albumin and 0.2% neomycinsulphate, pH 7.5, was used in all RIAs.

Plasma was extracted using octadecasilyl cartridges (SEP-PAK, Waters Inc, Milford, USA) as described for urine[6]. Generally 1 or 2 ml plasma were extracted and dried under vacuum in a Buchler vortex evaporator and kept stored at −70 °C until assayed.

For the RIA of AVP an antiserum kindly donated by Dr P. Baylis, Newcastle[7], was used. It has a crossreactivity with DDAVP < 0.01% and OT < 0.001%. The OT antiserum was obtained from Dr I.C.A.F. Robinson, London, England[8]. This antiserum crossreacts negligibly with AVP and DDAVP.

The antiserum for DDAVP was developed at Ferring, Malmö, using Dutch rabbits for immunizations. Antigen was produced by coupling NH_2-8-D-AVP to thyroglobulin by the carbodiimide method[9]. The conjugate was then mixed with Freund's complete adjuvant (Difco) and injected intradermally at multiple sites following the scheme of Sofroniew[9]. The best antiserum, designated ADA'6, had a titer of 1:100,000. The detection limit, defined as the smallest amount needed to displace zero binding by 2SD of the assay, is 1.5 ± 0.2 pmoles/l. Crossreactivity with OT and AVP is less than 0.008%. Immunoidentity of standard and plasma extracted DDAVP was assessed with HPLC using a Lichrosorb RP 18 Column (Merck, Darmstadt, West Germany). The mobile phase was 15% acetonitrile/85% 0.1 M ammoniumacetate, pH 6.8. This system fully separates AVP, OT and DDAVP. Eluted fractions were dried and stored at −50 °C until assay.

A rat was given an i.v. injection of 200 pmoles of DDAVP/kg. After 1 h the rat was decapitated and blood collected and treated as described above. In order to establish the specificity of plasma DDAVP the extract was dissolved in 100 µl distilled water and chromatographed. A standard solution of DDAVP was then subjected to HPLC under identical conditions and the two chromatograms were compared.

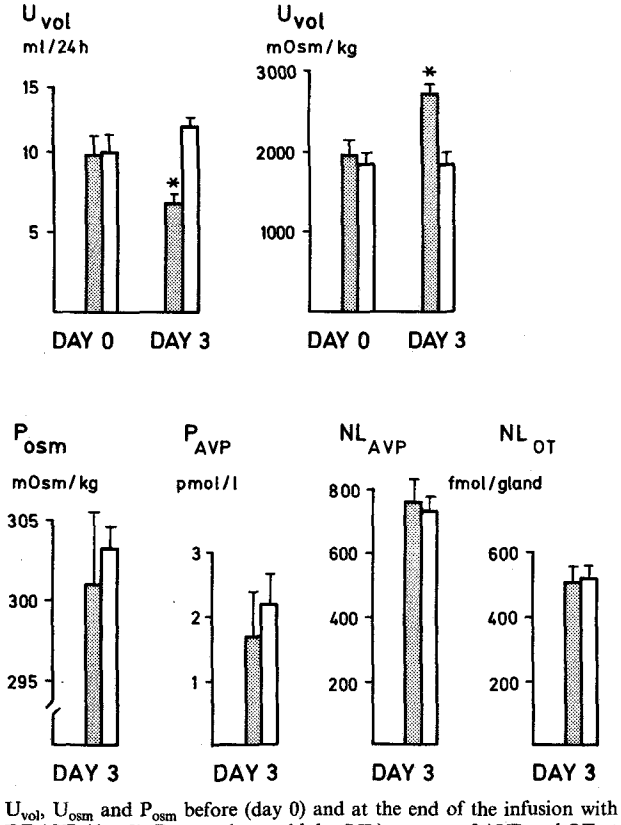
Recovery of DDAVP in the extraction method was determined by measuring a plasma pool containing 6.4 pmoles/l (n = 10). RIAs were performed using the conditions described for AVP by Möhring and Möhring[10]. Inter- and intraassay coefficients of variation were 8.1% and 18% respectively. Neural lobe extracts were not further extracted but diluted appropriately with RIA buffer.

Results are expressed as means ± SEM. Statistical significance between experimental groups are assessed by Student's t-test.

Results. The specificity of the RIA for DDAVP was determined with HPLC of plasma from a rat injected with 200 pmoles/kg b.wt. No interference from plasma material other than DDAVP could be detected. Recovery of DDAVP from the plasma pool was 87 ± 2% (n = 10).

The effects of prolonged DDAVP infusion are shown in the figure. As expected U_{vol} was decreased and U_{osm} increased after 3 days of infusion. The differences were statistically significant ($p < 0.005$) both when compared to values obtained before the infusion and to values from control animals infused with 0.9% NaCl only. Despite the induced antidiuretic state of the animals no changes were observed in plasma osmolality (P_{osm}).

Mean plasma concentrations of DDAVP on day 3 of the infusion were 30.4 ± 1.7 pmoles/l; however, AVP levels (P_{AVP}) remained unaffected when compared to those of control animals. In both the experimental group and in the control group OT in plasma fell below the detection limit of the assay, 2.5 pmoles/l. The neural lobe content of AVP and OT after 3 days of constant DDAVP infusion did not differ from the amounts of peptide extracted from the control rats.



U_{vol}, U_{osm} and P_{osm} before (day 0) and at the end of the infusion with DDAVP (day 3). P_{AVP} and neural lobe (NL) content of AVP and OT at the end of DDAVP infusion (day 3). Hatched bars = DDAVP infused rats; open bars = control animals infused with 0.9% NaCl. Means ± SD. Differences between groups were assessed with Student's t-test and $p < 0.005$ is indicated with an asterix.

Discussion. The present RIA for DDAVP is to our knowledge the first using a specific antiserum. It was found to be highly sensitive and specific especially in relation to the endogenous hormones AVP and OT.

Normal plasma concentrations of AVP were found to be approximately 2 pmoles/l. Considering that the antidiuretic potency of DDAVP is about three times that of AVP, plasma values of DDAVP of 30.4 ± 1.7 pmoles/l obtained during the infusion therefore mean that the experimental animals must have been in a maximal state of antidiuresis during the study period. The changes in U_{vol} and U_{osm} under these conditions were rather modest, i.e. less than 50%, indicating that rats under normal conditions with free access to water are close to being in maximal antidiuresis. This may explain the finding that plasma osmolality after 3 days of DDAVP infusion was not different from that of control animals showing that no significant water retention took place.

Neither plasma AVP nor neural lobe contents of AVP and OT were affected by DDAVP infusion, indicating an unchanged rate of release of AVP from the pituitary. This is to be expected since plasma osmolality, the primary stimulus for osmoreceptor function, was similar in experimental and control animals. It can therefore be concluded that prolonged infusion of DDAVP in rats does not interfere with the function of the hypothalamo-neurohypophysial system, neither as to biosynthesis nor as to release of AVP.

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0014-4754/85/070933-03$1.50 + 0.20/0

Oenocyte and prothoracic gland activity in *Manduca sexta* under varying photoperiod and light conditions*

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Summary. Manduca sexta larvae were subjected to diapause-inducing and diapause-preventing photoperiods, using two types of fluorescents (Indorsun and Blacklight-blue). The oenocytes, prothoracic glands (PTG) and ecdysone levels were examined in 3-day-old 5th instar larvae, 2-day-old and 10-day-old pupae. Our results indicate that oenocytes and PTG cells tend to be more active under long photoperiods while oenocytes only are active under short photoperiods in pupae in diapause. UV light has a definite effect on oenocytes while PTG cells seem to be unaffected. Ecdysone and ecdysterone levels vary with PTG and oenocyte activity at the pupal stage. The significance of these findings is discussed.
Key words. Manduca sexta larvae; photoperiod; oenocytes; prothoracic gland; ecdysone; diapause conditions.

The use of histochemical and biochemical techniques, and of electron microscopy has contributed extensively to our understanding of the structure and function of oenocytes. It is now known that these cells play a role in hormonal metabolism, converting ecdysone to ecdysterone and, possibly, synthesizing ecdysterone[2,3]. The participation of oenocytes in cuticle formation was demonstrated by ^{14}C incubation in vitro and in vivo[4-6]. In the course of insect development, the ultrastructure of oenocytes has been shown to undergo significant changes[7-14]. Little, if no attention, has been paid to the participation of oenocytes in the phenomenon of diapause. Because the latter is under hormonal control, it would seem reasonable to assume that oenocytes have a role in the induction, maintenance and/or prevention of diapause[15]. By studying the changes taking place in oenocytes under changing photoperiod and light regimes it might be possible to get a better understanding of how diapause is controlled.

In the present study, we present evidence that the oenocytes of *Manduca sexta* undergo changes under diapause-inducing and diapause-preventing conditions and that such changes are synchronous with variations in prothoracic gland activity and ecdysone levels.

Materials and methods. Larvae of the tobacco hornworm, *Manduca sexta,* were reared under diapause-inducing (6L:18D) and diapause-preventing (18L:6D) photoperiod conditions at 26 °C on a synthetic diet[16]. Indorsun fluorescent tubes (Verd-A-Ray Corp.) were used emitting 25 W/m^2 at 20 cm. Larvae were also subjected to UV light conditions provided by Westinghouse blacklight-blue fluorescent tubes (0.4 W/m^2 at 20 cm). There were therefore four rearing conditions: 1) Indorsun short photoperiod: ISP; 2) Indorsun long photoperiod: ILP; 3) UV short photoperiod: UVSP; 4) UV long photoperiod: UVLP.

On the 3rd day following ecdysis to the 5th instar, five larvae from each rearing condition were frozen rapidly, cut into small sections and fixed in Carnoy's. After paraffin embedding, the material was sectioned at 8 µm and stained with Feulgen's reagent, with 1% light green as counterstain. The same procedure was followed with 2-day- and 10-day-old pupae.

Measurement of ecdysone levels in 3-day-old 5th instar larvae, 2-day- and 10-day-old pupae was carried out by high-performance liquid chromatography (HPLC) using a Beckman model 332 gradient system, with an ODS 5 reverse phase column (4.5 × 250 mm). Ecdysone and ecdysterone standards were supplied by Sigma Chemicals. The hemolymph was collected in vials placed in an ice bath and processed according to the method of Lafont et al[17]. An acetonitrile buffer system and gradient solution (20:80) was used for HPLC analysis of the ecdysones, the detection limit of which is 20 ng.

Results. 1) Oenocytes. Larval oenocytes of *M. sexta* exhibited slight histological differences under diapause-inducing and diapause-preventing conditions. Under ILP conditions the DNA positive material of the nuclei was not homogeneous and a large nucleolus sometimes present. Under ISP conditions, nucleoli were seldom seen.

There were clear-cut differences between oenocytes of larvae reared under UVSP and those reared under ISP conditions (fig. 1). UVSP oenocytes had abundant, dark-stained cytoplasm. The nuclei were smaller in UVSP oenocytes than in their ISP counterparts. UVSP oenocytes looked active as opposed to the 'exhausted' appearance of the irregularly shaped ISP oenocytes. While the oenocytes of 2-day-old developing pupae (ILP) were essentially similar to their larvae counterparts pupae in diapause (ISP) were characterized by irregularly shaped oenocytes with less dense cytoplasmic material.

10-day-old developing pupae (ILP) had two distinct populations of oenocytes (fig. 2): 1) the fading larval oenocytes and 2) the newly formed imaginal oenocytes.

2) Prothoracic glands. There were remarkable differences between larval PTG cells under ILP and ISP conditions (fig. 3). ILP cells were characterized by a smooth surface though they could be irregularly shaped. The nucleus had homogeneously distributed DNA, and prominent nucleoli.

Under ISP conditions PTG cells tended to be vacuolated. DNA positive material was unevenly distributed in the nuclei. Nucleoli were not visible.

There seemed to be no striking difference between PTG cells of larvae reared under UV light and under Indorsun conditions. They all had lightly staining cytoplasm, the peripheral striation of ISP cells being more pronounced. Nucleic material was distributed unevenly but stained strongly for DNA.