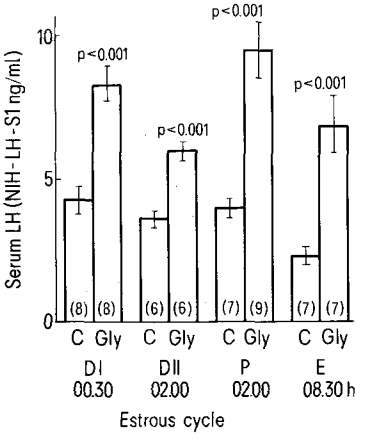
RH) in the middle hypothalamus significantly decreased during the critical period[8]. The present experiment was performed to investigate the effect of glycine on serum luteinizing hormone (LH) in adult female rats.

Materials and methods. Experiments were carried out on adult female rats, weighing 250–300 g. Laboratory chow and tap water were provided ad libitum. The animals were kept under reversed lighting (light from 22.00 h–10.00 h) for 30 days before the starting to take vaginal smears. Under our laboratory conditions, the critical period is around 05.00 h–08.00 h on proestrus[9]. Daily vaginal smears were taken before 23.45 h until autopsy. Each of the rats selected had shown at least 3 consecutive 4-day estrous cycles before the cycle when it was used. Glycine was dissolved in 0.9% NaCl. 200 mg of glycine or 0.9% NaCl in a volume of 1 ml was injected i.p. into each of the 4-day cyclic rats at various times throughout the estrous cycle. 20 min after injection, the animals were exsanguinated by decapitation. After centrifugation the serum was kept frozen at −20 °C until the day of assay. LH was measured by radioimmunoassay using NIAMDD reagents. The samples were assayed in duplicate with an intra-assay coefficient of variation of less than 5%. All samples from each experiment were run in the same radioimmunoassay.



Serum LH levels 20 min after injection of 0.9% NaCl or glycine at various stages of the estrous cycle. Vertical lines indicate ±SEM, and the number of rats is given in parentheses. p-values are for control vs experimental in each experiment. Abbreviations are as follows: C = 0.9% NaCl, Gly = glycine, D_I = diestrus I, D_{II} = diestrus II, P = proestrus, E = estrus.

Results and discussion. The results are shown in the figure. At all stages of the estrous cycle, the i.p. injection of 200 mg of glycine produced significant increases in serum LH levels ($p < 0.001$). However, only the small changes in the response at different stages could be observed. The response was minimal on diestrus II and maximal on proestrus. The results of the present study demonstrate that the acute administration of glycine elevates serum LH levels in adult female rats. Tada et al.[10] observed that glycine injected i.p. passed the blood-brain barrier. The present results together, with the previous observations[7,8], suggest the possibility that the site of action of glycine may be within the middle hypothalamus and glycine may play a role in regulating the LH-RH release. However, Ondo et al.[11] demonstrated that serum LH levels did not increase following the intraventricular or intrapituitary administration of glycine. The discrepancy between our present results and the results of Ondo et al. may depend on the different uptake site of glycine following the different administration. Further experimentation is required to find the site in the central nervous system where glycine causes the increased LH release.

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Evidence for in vitro release of neurophysin by the rat pineal gland

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Summary. Cultured pineal ependymal cells from rat fetuses release into their incubation media immunoreactive neurophysin. The presence of neurophysin was assessed by radioimmunoassay. The culture medium was found to contain 440 pg neurophysin per mg protein.

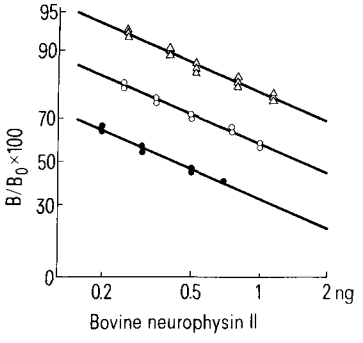
We have observed immunoreactive neurophysins in both bovine[1] and human[2] pineal glands. 2 immunoreactive neurophysins, distinguished by their electrophoretic mobility and their antigenic properties, were present in each species. The pineal neurophysins also share many characteristics with the pituitary neurophysins of the same species. The present communication reports the presence of immunoreactive neurophysin in the media of cultured pineal cells from rat fetuses.

Materials and methods. Lyophilized culture media from pineal cells of rat fetuses[3] were supplied by Dr Pavel. This method is detailed elsewhere[3] and is summarized below. The cells corresponding to each gland were suspended in 1 ml of Hank's medium supplemented with 10% calf serum and 2.5% N16 medium. On day 8, when the cell cultures were established, the media were first changed and thereafter they were changed every 5 days. Lyophilized media from 18-day-old fetal pineal cells representing 6.2 mg protein per ml were dissolved in 0.9% NaCl. The medium without cells served as control. The neurophysin was detected by radioimmunoassay (RIA) with an antibody directed against bovine neurophysin II (bNII). This antibody displays a low specificity in that it recognized not only bNII but also one of the neurophysins from man and rat.

The following procedure for RIA was applied. To 0.05 M Tris-HCl buffer pH 7.5 containing 0.5% of bovine serum albumin, 10 to 100 µl of the samples and 10 µl of the antibody (final dilution 1:20,000) were added. After 2 days incubation at 4 °C the labelled [^{125}I]-bNII was added (final volume of 0.5 ml). The antibody-bound [^{125}I]-bNII was separated from free [^{125}I]-bNII by precipitation with 3% zirconyl phosphate[5].

Results. 19 media of incubated pineal cells from rat fetuses were assayed for the presence of neurophysin by RIA. We have found that these samples displaced the labelled pituitary bNII in the same manner as the homogenate of rat posterior pituitary lobe.

Expressed in pg neurophysin per mg proteins, we obtained a mean value of 442±103 (SD) pg/mg. This is significantly higher than the mean value of the control samples: 229±12 (SD) pg/mg (probably due to neurophysin present in calf serum). Some caution is required in the interpretation of the quantiative aspect of these data, as they are based on RIA with highly purified bovine pituitary neurophysin without knowledge of the relative affinity of the rat neurophysin to the antibody used. The dilution curves performed with a pool of the samples are parallel to the standard curve with bNII and the dilution curve of rat pituitary homogenate (figure).



Dilution curves of cell culture media from pineal glands of rat fetuses (△), and of crude homogenate of rat posterior pituitary gland (●), in comparison with the standard curve of bovine neurophysin II (○) determined by radioimmunoassay (logit-log plot). The quantity of culture media ranged from 0.2 to 2 mg and that of posterior pituitary homogenate from 20 to 70 µl (1 lobe in 0.5 ml).

Discussion. The demonstration by Pavel[4] that the mammalian pineal gland contained vasotocin prompted our own studies, in which we detected neurohormone carrier proteins first in the bovine[1] then in the human gland[2] and now in the media of cultured ependymal cells from rat fetuses. The present data, in which the quantity of neurophysin in incubated cell media was significantly greater than that of control, suggest that the pineal gland is capable of releasing in vitro neurophysin, which probably represent the carrier protein of vasotocin. Pavel has already demonstrated that rat and human pineal cells can synthesize vasotocin in vitro. Our demonstration that the pineal gland releases neurophysin is another piece of evidence indicating that this gland contains, releases and probably synthesizes the complete set of at least 1 neurohormone and its carrier protein or remnant of precursor form. The physiological significance of this activity remains to be established.

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Role of the nerve cord in the control of prothoracic glands in *Galleria mellonella* L.

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Summary. Certain lesions of the nerve cord in postfeeding larvae impede pupation by altering the proprioreceptive input, which modulates the prothoracicotropic activity of the brain. Direct influence of ventral ganglia on prothoracic glands is negligible.

Pupation of the wax moth is delayed or prevented after transection of the ventral nerve cord or destruction of ventral ganglia[1-4]. Edwards[2] concluded that such lesions blocked the activation of prothoracic glands (PTG) by the brain, but Alexander[3] assumed that PTG were inhibited via the suboesophageal ganglion. Since we found that also the thoracic ganglia may play a role in the control of PTG[5], we have re-examined significance of different ganglia for the onset of metamorphosis.

Material and methods. We used our stock culture of *Galleria* and employed standard surgical techniques[5]. The age of larvae was known within ±8 h after preceding ecdysis. Most experiments were performed with wandering larvae (132 h after the last larval ecdysis). The operations involved removal of the brain, suboesophageal (SG), thoracic (T_1, T_2, T_3) and abdominal (A_1–A_8) ganglia and sections of the connectives. Control insects were subjected to injuries identical to those inflicted during operations but the nerve cord was not touched. Each experiment was performed on 15–25 larvae. The percentage of pupating insects and the length of time between operation and ecdysis (mean time ± SD in days) were recorded.

Results and discussion. Larvae with severed nerve cord often shrank anteriory to the cut whereas their posterior segments appeared normal or slightly swollen[2]. Sectioning reduced mobility and spinning of larvae, in particular when the cord was cut in the region between T_2 and A_3. In accordance with previous authors[2-4], we found that this treatment inhibited pupation in a considerable number of the larvae and delayed pupal ecdysis in the remainder