Effect of age, ambient temperature, and exposure to hormone on the posteclosion diuretic response of the monarch butterfly

R. M. Dores and W. S. Herman

University of Colorado Health Sciences Center, Department of Physiology, C240, 4200 E. 9th Ave., Denver (CO 80262, USA), and Department of Genetics and Cell Biology, University of Minnesota, St. Paul (MN 55108, USA), 27 October 1980

Summary. The monarch butterfly loses sensitivity to the posteclosion diuretic hormone of this species within 12 h of eclosion. This de-sensitization can be accelerated by exposure to the diuretic hormone, and decelerated by both hormone deficiency and reduced temperature.

A previous study[1] has indicated that posteclosion diuresis in the monarch butterfly, *Danaus plexippus* (L), is regulated by a diuretic hormone (DH). This hormone is a water-soluble, heat-stable, trypsin-sensitive, 3000-dalton polypeptide which is localized in the cephalic endocrine system of this species[1]. Hormonal regulation of posteclosion diuresis has also been observed in the cabbage white butterfly, *Pieris brassicae*[2], and a comparable system has recently been reported for the painted lady butterfly, *Vanessa cardui*[3]. The present study will focus on the loss of sensitivity to the DH which has been previously noted in older adult monarchs[1]. Experiments were conducted to determine the effects of age, ambient temperature, and prior hormone exposure on the de-sensitization of adult monarchs to this hormone.

Methods of adult procurement, neck-ligature, hormone extraction and injection, weight determination, and the composition of the insect saline used in these studies, have all been described[1]. Measurements of diuresis at eclosion (i.e., 0 h) were made on both intact and neck-ligatured monarchs that were either uninjected, injected with DH extract (1 head equivalent/25 µl), or injected with 25 µl of distilled water. For time intervals of 12 h to 30 days posteclosion, the diuretic response was measured by determining the weight loss following an injection of 300 µl of insect saline that did or did not contain DH. The dose of DH injected for these experiments was also 1 head equivalent. All responses were calculated as net weight loss, which was the experimental animal weight loss minus the mean control weight loss, in mg, over a 3-h period. Details of the above procedures are presented elsewhere[1]. All data are presented as mean ± SEM; statistical statements are based on the use of the Student's t-test.

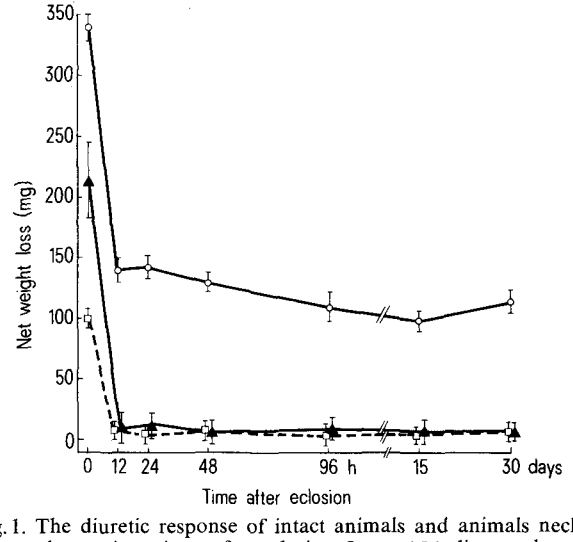

Fig. 1. The diuretic response of intact animals and animals neck-ligatured at various times after eclosion. Intact (○); ligatured + extract (▲); ligatured + saline (□). The data is presented as mean ± SEM (n = 6).

The influence of age on the diuretic response of adult monarchs was examined at specific intervals over a 30-day period (fig. 1). Animals left intact and injected with saline showed a conspicuous diuretic response throughout this time period. In addition, as previously shown[1], animals neck-ligatured at eclosion and immediately injected with DH extract also showed a significant response over the saline injected neck-ligatured controls. By contrast, animals neck-ligatured at 12 h posteclosion and all subsequent times intervals thereafter did not respond to DH extract injections (fig. 1). These results indicate that the ability of the monarch to respond to the DH is limited to a short interval immediately after eclosion and is essentially eliminated by 12 h posteclosion.

Next the effect of ambient storage temperature on the posteclosion loss of sensitivity to the DH was examined. In these experiments butterflies were neck-ligatured immediately after eclosion to prevent exposure to intrinsic DH. These animals were then stored at either 25 °C or 10 °C and tested at various times after eclosion for sensitivity to DH

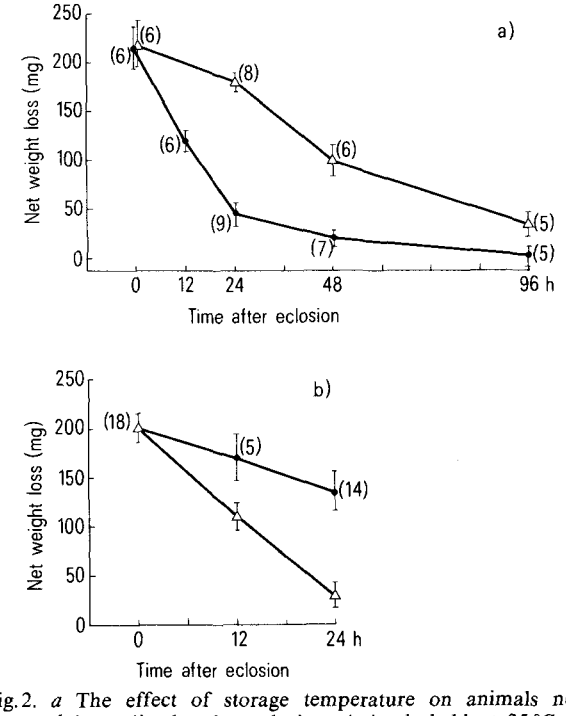

Fig. 2. *a* The effect of storage temperature on animals neck-ligatured immediately after eclosion. Animals held at 25 °C (●) and 10 °C (△). The number of animals assayed at each time period is represented by the figures in the brackets. *b* The effect of single vs multiple injections of DH on the diuretic response of animals neck-ligatured at eclosion and held at 10 °C. (△) represents animals which received multiple dose of DH, while (●) represents animals which received a single dose of DH extract. For the multiple-dose experiment, n = 11. For the single-dose experiment n is represented by the figures in the brackets. The data is represented as mean ± SEM.

extracts (fig. 2a). These manipulations in the case of the 25 °C animals extended the period of responsiveness to the hormone to 12 h posteclosion. Furthermore, the animals stored at 10 °C showed an even longer period of responsiveness to the DH and were capable of a strong diuretic response to the hormone at 48 h posteclosion. These experiments indicate that in the apparent absence of intrinsic DH, there is a temperature dependent loss of sensitivity to DH injections. The delayed loss of DH sensitivity noted in neck-ligatured animals stored at 10 °C could prove useful as an assay system for future studies.

Since removal of intrinsic DH coupled with storage at 10 °C enhanced the period of responsiveness in neck-ligatured animals, the effect of multiple injections of DH on animals neck-ligatured and held at 10 °C was examined. These animals were divided into 2 groups. Each animal in the 1st group was injected with DH extract at 0, 12, and 24 h posteclosion. Animals in the 2nd group were given a single injection at either 0, 12, or 24 h posteclosion. As indicated in figure 2b animals given multiple injections began to show a decline in sensitivity to the DH extract after the 2nd injection, furthermore, by 24 h posteclosion the sensitivity of these animals to the DH had been eliminated. By contrast, animals given single injections over this time period still respond to the DH injection.

The above experiments demonstrated that, even though DH is present in all adult monarchs[1], the sensitivity of the butterfly to DH is restricted to a short period of adult life. Furthermore it appears that this loss of sensitivity to the DH is both temperature-dependent and DH-sensitive. It therefore seems conceivable that this system may represent an invertebrate example of the desensitization phenomena which have been commonly reported in vertebrate systems[4]. If so, it would be of interest to obtain additional data concerning the molecular details of this process in the monarch.

1 R.M. Dores, S.H. Dallmann and W.S. Herman, J. Insect Physiol. *25*, 897 (1979).
2 S.W. Nicolson, J. Insect Physiol. *22*, 1347 (1976).
3 W.S. Herman and S.H. Dallmann, J. Insect Physiol., in press (1981).
4 K.J. Catt, J.P. Harwood, G. Aguilera and M.L. Dufau, Nature *280*, 109 (1979).

Effects of prolactin (PRL) on gonadotropin release in mice with congenital PRL deficiency[1]

A. Bartke, T.M. Siler-Khodr and F. Bex

Department of Obstetrics & Gynecology, The University of Texas Health Science Center, San Antonio (Texas 78284, USA), and Endocrine Section, Wyeth Laboratories Inc., P.O. Box 8299, Philadelphia (Pennsylvania 19101, USA), 30 July 1980

Summary. In prolactin (PRL)-deficient male dwarf mice, treatment with PRL stimulates the release of FSH without affecting plasma LH levels. We now report that this effect of PRL is not mediated by the testes and that PRL does not modify FSH or LH release in female dwarf mice.

In addition to regulating its own release[2], prolactin (PRL) has been shown to suppress secretion of pituitary gonadotropins in a number of mammalian species[3]. However, in male dwarf *(dw/dw)* mice with hereditary PRL deficiency[4], treatment with ovine PRL or with PRL-secreting ectopic pituitary grafts produced significant increase in plasma FSH levels[5]. We now report that this unexpected effect of PRL in dwarf mice is not mediated through the testes, and that PRL does not modify gonadotropin release in female dwarf mice.

Dwarf mice *(dw/dw)* were raised in our own colony and maintained in a room with 14 h of light: 10 h of darkness with continuous access to food and water. 16 males were castrated and 9 of them were given a transplant of 1 pituitary gland each from a normal (non-dwarf) adult female under the renal capsule. 36 female dwarf mice were divided into 4 treatment groups as follows: 12 were given pituitary grafts, 9 were sham-operated, 9 were injected s.c. daily with 125 µg ovine PRL (NIH-P-S12) in 0.05 ml saline, and 6 were injected with saline alone. 2 weeks after the onset of these treatments, blood samples were collected by cardiac puncture under ether anesthesia and the animals were sacrificed. Pituitary glands were homogenized in cold 0.01 M phosphate buffered saline, pH = 7.6. The concentration of FSH and LH in plasma and pituitaries was determined by NIAMDD rat FSH radioimmunoassay and ovine: ovine LH radioimmunoassay[6] and expressed in terms of NIAMDD rat standards FSH-RPI and LH-RPI. These procedures have been validated for use in the mouse[7]. Results obtained in saline-injected and sham-operated controls were combined for statistical analysis.

In castrated pituitary-engrafted male dwarf mice, plasma FSH levels were twice as high as in castrated controls ($p < 0.025$) (table 1). In contrast, plasma LH levels were nearly identical in the 2 groups. Treatment of female dwarf mice with PRL or grafts had no effects on plasma FSH and

Table 1. Effects of pituitary grafts on FSH and LH levels in castrated male dwarf *(dw/dw)* mice

	Control (7)	Grafts (9)
Plasma FSH (ng/ml)	824 ± 240	1708 ± 198*
Plasma LH (ng/ml)	119 ± 61	104 ± 35
Pituitary FSH (ng/mg)	3786; 2836	4930; 4304
Pituitary LH (ng/mg)	2786; 1391	1448; 1530

Values are means ± SE for individual plasma samples or values obtained in pools of 3-4 pituitaries. Number of animals in parenthesis. *Significantly different from controls; $p < 0.025$.

Table 2. Effects of prolactin (PRL) and pituitary grafts on FSH and LH levels in female dwarf *(dw/dw)* mice

	Control (15)	PRL (9)	Grafts (12)
Plasma FSH (ng/ml)	120 ± 16	144 ± 3	108 ± 8
Plama LH (ng/ml)	24 ± 10	43 ± 16	< 8
Pituitary FSH (ng/mg)	3058 ± 485	4203 ± 1234	2401 ± 296
Pituitary LH (ng/mg)	9069 ± 1125	6605 ± 712	4540 ± 631

Samples from 3 mice were pooled for each determination and the results are reported as means ± SE. Number of animals in parenthesis. Significance of the differences in the text.