Endocrine regulation of the bursa copulatrix and receptacle gland of *Danaus plexippus* L. (Lepidoptera:Danaidae)

W. S. Herman

Department of Genetics and Cell Biology, University of Minnesota, St. Paul (Minnesota 55108, USA), 12 October 1981

Summary. Significant growth occurs in the bursa copulatrix and receptacle glands of female Monarch butterflies held in summer conditions after adult emergence. Removal of the corpora allata prevents this growth, but normal development occurs if juvenile hormone is injected into females lacking corpora allata.

Earlier research has demonstrated pronounced posteclosion development of the ovaries (OV) and colleterial glands (CG) of the adult female Monarch butterfly, *Danaus plexippus*, and has also shown that this organ growth is regulated by juvenile hormone (JH)[1-3]. In these previous studies, little attention was given to the 2 other major portions of the female Monarch reproductive tract, i.e., the bursa copulatrix (BC) and the receptacle gland (RG). No precise description of the posteclosion growth of these 2 organs was provided and, even though JH appeared to occasionally stimulate growth of one or both organs[1-3], the possibility that JH also regulated the Monarch BC and RG was not fully explored. I therefore decided to examine the posteclosion growth of both organs, and to test the effects of appropriate endocrine manipulations on their development in young adults. I believed that such investigations might elucidate the role, if any, of JH in the regulation of Monarch BC and RG, and thereby provide data of use in our attempts to obtain a more complete understanding of the role of JH in females of this species. These studies have now been completed; they provide strong evidence that the BC and RG are both regulated by JH in adult female Monarch butterflies.

The animals used in these studies were obtained from larvae reared outdoors on milkweed, *Asclepias syriaca*, in June and July. Some females were dissected on the day of emergence, while others were either experimentally manipulated or left intact and then held at 25 °C with a 16 h photophase for 4 or 5 days prior to dissection. Intact animals were fed 30% honey daily, while ligated females were maintained by daily injections of 10% glucose. The Ayerst mixture of JH I isomers (AJH), used in previous work on this species[1-3], served as hormone in these studies. The anatomy of the Monarch female reproductive tract, the terminology applied to various portions of the tract, and procedures for dissection, organ weighing, allatectomy, neck-ligation, and hormone injection (with mineral oil as solvent) are detailed elsewhere[1-3]. Dry weights were determined by holding individual organs at 100 °C until constant values were obtained. The data were subjected to Students t-test, and the term significant in this report refers to statistical significance in that test at the level of $p < 0.05$.

I initially attempted to demonstrate significant increases in BC and RG wet and dry weights after eclosion in untreated females (see table 1). The data showed that BC wet and dry weights both increased significantly from day 0 to day 8, but significantly higher weights were not observed thereafter. Wet and dry weights of the RG both showed significant elevation by day 4, but thereafter both weights obviously decreased. By 15 days after emergence BC weights were still elevated over the day 0 level, but RG weights approximated those observed at eclosion. The behavior of the RG was totally unlike that observed in any other portion of the female reproductive tract[3].

In view of the above results, the effects of allatectomy and neck-ligation, with or without subsequent AJH replacement, were examined in several experiments. The combined data from these studies are summarized in table 2, which also includes for comparison purposes the effects of the various treatments on OV and CG weights.

Once again, in different animals from those used in table 1, significant growth was noted in the 1st 4–5 days after eclosion in both the BC and RG. When compared to the OV and CG, this development was not quantitatively impressive, i.e., OV and CG wet weights exhibited increases of 18.0 and 8.0 times, respectively, the day 0 value, while BC and RG wet weights increased only 1.3 and 2.1 times, respectively, during the same period.

Allatectomy prevented normal development of both the BC and RG (see table 2). Injection of AJH into allatectomized animals promoted significant weight increases in both BC and RG, and the values obtained from such females were comparable to those obtained from sham-operated insects. As expected from previous studies[1-3], allatectomy also prevented development of the OV and CG and injections of AJH into animals lacking the corpora allata led to pronounced weight gain in both organs.

The neck-ligation experiments produced results comparable to those obtained in allatectomized females. Injections of hormone solvent into ligated animals resulted in female organs that were not substantially different from those observed on day 0, except for an inexplicable 22% increase in RG weight. Injections of AJH resulted in significant increases (over the solvent injected control values) in the wet weights of all pertinent organs, and in the case of

Table 1. Weight changes in Monarch female reproductive organs after eclosion

Days after eclosion	Bursa copulatrix		Receptacle gland	
	Wet weight	Dry weight	Wet weight	Dry weight
0	9.9±0.3	(17) 1.8±0.1	1.7±0.1	(15) 0.4±0.0
4	12.9±0.2	(13) 3.6±0.1	4.0±0.2	(13) 0.9±0.0
8	14.4±0.5	(12) 4.9±0.2	2.6±0.1	(12) 0.7±0.0
15	14.6±0.6	(5) 5.5±0.3	1.6±0.1	(5) 0.5±0.0

Data presented as mean mg wet weight or dry weight ± SEM; n in parentheses.

Table 2. Effect of juvenile hormone on posteclosion BC and RG development

Treatment		Organ wet weight			
		BC	RG	OV	CG
Intact					
Day 0	(10)	9.8±0.3	1.8±0.1	2.2±0.1	0.7±0.1
Day 4–5	(18)	12.6±0.3	3.8±0.3	39.7±3.4	5.6±0.6
Allatectomy					
+ mineral oil	(6)	9.7±0.3	1.8±0.0	4.5±0.5	0.9±0.1
+ 100 µg AJH	(8)	11.0±0.4	3.0±0.3	24.0±2.8	3.0±0.3
sham operated	(8)	11.5±0.4	2.6±0.2	29.5±4.9	4.0±0.6
Neck ligation					
+ mineral oil	(18)	9.6±0.2	2.2±0.1	2.2±0.1	0.8±0.0
+ 30 µg AJH	(16)	10.3±0.2	3.0±0.1	4.3±0.2	2.1±0.2
+ 100 µg AJH	(14)	10.8±0.3	3.4±0.2	9.2±1.3	3.0±0.3
+ 300 µg AJH	(18)	11.0±0.4	3.5±0.1	13.6±1.5	4.1±0.3

Data presented as mean ± SEM in mg wet weight; n in parentheses; abbreviations defined in text; all experimental animals held 4–5 days at 25 °C.

all organs except the BC the increases were significantly higher in females given larger doses of AJH.

Thus, the data showed that the normal posteclosion growth of both the BC and RG occurring in intact female adult Monarchs was prevented by procedures removing the source of JH, i.e., by allatectomy or neck-ligation. In addition, the data showed that in females lacking the source of JH apparently normal growth of both glands was produced by injections of material (AJH) known to possess high JH activity in this species[1-3]. Finally, the BC and RG changes occurring in response to AJH seemed to be dose-related, and they clearly paralleled those observed in 2 female organs (the OV and CG) known to be regulated by JH[1-3]. It therefore appears that the posteclosion growth of the BC and RG of this species, like that of the OV and CG, is regulated by JH. Apparently, the relative importance of this regulatory mechanism declines with age, since studies on female Monarchs several weeks or months after eclosion have failed to provide conclusive evidence for JH mediated regulation of the BC and RG[3].

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Prolactin and growth hormone do not interfere with the response of mouse testes to hCG in vitro[1]

A. Bartke and Susan Dalterio

Departments of Obstetrics & Gynecology and Pharmacology, The University of Texas Health Science Center, San Antonio (Texas 78284, USA), 6 August 1981

Summary. In incubations of decapsulated mouse testes with a maximally stimulating dose of hCG, the accumulation of testosterone was not affected by the addition of PRL at concentrations from 0.1 to 50 µg/ml or GH at concentrations from 0.5 to 25 µg/ml.

Hypogonadism in hyperprolactinemic men is most likely due to inhibition of LHRH release and consequent gonadotropin deficiency[2,3], but the possibility of direct inhibitory effects of PRL on testicular steroidogenesis has not been eliminated. Experimental induction of hyperprolactinemia in mice is associated with a reduction in the number of testicular LH receptors and in the responsiveness of the testes to hCG stimulation in vitro[4]. These effects are thought to be due to a chronic elevation of peripheral LH levels which accompanies hyperprolactinemia in this species, but the possibility remains that PRL may also exert direct inhibitory effects on the testes. The present work was undertaken to determine whether the acute steroidogenic response of the testes to gonadotropin stimulation in vitro is affected by the presence of PRL in the incubation media. Because of the overlap of biologic activities of PRL and GH, the effects of the latter hormone in the same system were also examined.

The experiments were conducted using procedures originally developed by Dufau et al.[5] and by Van Damme et al.[6]. Adult random-bred mice were killed by cervical dislocation; testes were removed, decapsulated, and incubated for 4 h in 2 ml Krebs Ringer bicarbonate buffer containing glucose (1 mg/ml) and various doses of hCG (A.P.L. chorionic gonadotropin, Ayerst Laboratories) in a Dubnoff metabolic shaker at 32 ± 1 °C (under O_2:CO_2 (95:5). Ovine PRL (NIH-P-S12) and ovine GH (NIH-GH-S11) were dissolved in physiologic saline containing a few drops of 0.1 N NaOH, diluted and added to incubations to achieve the desired concentrations. Control incubations contained contralateral testes from the same mice and identical amounts of hCG, saline and NaOH. Testosterone (T) radioimmunoassays were performed directly on aliquots of the incubation medium[6-8].

In pilot studies, addition of 0.1–10 µg oPRL/ml to mouse testes incubated with 12.5 mIU hCG/ml caused a significant and dose-related increase in the accumulation of T in the media. However, addition of 20 ng FSH plus 5 ng LH/ml (maximal amounts of FSH and LH which could be present in 1 µg of NIH-P-S12) also caused a pronounced stimulation of T accumulation. In order to eliminate the influence of gonadotropins present in the oPRL preparation, an approach similar to that developed by Johnson and Ewing[9] was utilized, namely all further experiments were conducted in the presence of maximally stimulating amounts of hCG. The dose of hCG maximally effective in this system was determined to equal 100 mIU hCG/ml. In the presence of this dose of hCG, addition of oPRL at concentrations of 0.1–50 µg/ml had no significant effect on T production (table). Similarly, addition of 0.5, 5 or 25 µg GH/ml to incubations containing 100 mIU HCG/ml had no effect on the accumulation of T in the media. In additional experiments, effects of 2.5 µg oPRL/ml were examined in the presence of a higher and a lower dose of hCG. In incubations containing 50 mIU hCG/ml, this dose of oPRL caused a significant stimulation of T accumulation (504 ± 47 vs 818 ± 76 ng/ml; $p < 0.001$; $n = 18$), while in the presence of 200 mIU hCG/ml it was without effect (966 ± 69 vs 1065 ± 100 ng/ml; $n = 9$).

Thus the present findings argue against the possibility that alterations of testicular function in hyperprolactinemic

Effects of prolactin (PRL) and growth hormone (GH) on the production of testosterone by decapsulated mouse testes incubated with 100 mIU of human chorionic gonadotropin (hCG)/ml. Contralateral testes from the same animals incubated with hCG alone served as controls. The results represent mean (± SE) concentration of testosterone in the medium at the end of a 4-h incubation

Treatment	Concentration of testosterone (ng/ml)		
	Number	Controls	Treated
PRL; 0.1 µg/ml	12	1240 ± 105	1232 ± 67
PRL; 0.5 µg/ml	12	1201 ± 86	1167 ± 87
PRL; 2.5 µg/ml	15	923 ± 126	1026 ± 122
PRL; 5.0 µg/ml	27	843 ± 99	1013 ± 79
PRL; 25.0 µg/ml	8	1867 ± 208	1973 ± 164
PRL; 50.0 µg/ml	6	1384 ± 58	1396 ± 151
GH; 0.5 µg/ml	6	1061 ± 123	998 ± 104
GH; 5.0 µg/ml	6	1120 ± 99	1094 ± 117
GH; 25.0 µg/ml	9	1369 ± 153	1465 ± 113