Effects of Chronic Melatonin and Saline Injections on Pituitary Adrenal Secretion

The results of a number of studies[1-3] have suggested a functional relationship between the pineal gland and the pituitary adrenal axis.

In the present series of experiments, the effects of injected melatonin on pituitary and plasma ACTH content was determined, and corticosterone concentration in the plasma and the adrenal gland was measured. Appropriate controls allowed for assessing the effects of the chronic stress of repeated injections.

Forty male rats (Long-Evans strain Simonson) housed individually were used. The experimental room had a 12 h light/dark cycle; experimentation was conducted approximately 3 h after the onset of light. The experiment began when the animals were 80 days of age, and extended over 10 consecutive days. The animals were randomly divided into 4 equal groups; (1) acute melatonin-animals were weighed daily on days 1–10 but were injected with melatonin on day 10 only; (2) acute vehicle-animals were treated identically as described above but were injected on day 10 with the vehicle; (3) chronic melatonin-animals were weighed and injected with melatonin on days 1–10; (4) chronic vehicle control – as group (3) – but were injected with vehicle on days 1–10.

Melatonin (1 mg/kg) was administered s.c. in a volume of 0.1 ml/100 g body weight. The melatonin (Regis Chemical) was dissolved in ethanol and further diluted with saline to give a 1% solution of ethanol in saline. The 1% ethanol vehicle was used for the control injections.

Thirty minutes after injecting the animals on day 10, they were removed from their home cage and immediately placed in a jar saturated with ether for 1 min. The jugular vein was exposed and 0.4 ml of blood was drawn into a heparinized syringe; the procedure was accomplished within 90 sec. Due to the time lag of the rise in peripheral plasma corticosterone levels after the onset of a 'stressor'[4], this initial sample gave a measure of the animals' plasma steroid levels 30 min after the injections and provided a comparison level for the subsequent adrenocortical response to ether and surgical stress. After 15 min had elapsed the animals were decapitated and a trunk blood sample was obtained. The anterior pituitary glands were immediately removed. The second blood sample provided a measure of the plasma steroid concentration following exposure to ether and jugular venisection. Plasma corticosterone concentration was determined on individual samples according to the fluorometric micromethod of GLICK et al.[5].

The post-ether plasma samples were pooled and assayed for ACTH content. The anterior pituitaries were extracted in 0.1 *N* HCl and stored at −12 °C until assayed for ACTH content[6]. The bioassay for plasma ACTH previously described by VERNIKOS DANELLIS et al.[7] was used. Adrenocorticotropin concentration was expressed as mU ACTH/100 ml plasma or mU ACTH/mg pituitary tissue.

The results are shown in the Table. Analyses of the effects of chronic treatment with melatonin on body and anterior pituitary weights yielded no significant differences between the melatonin treated animals and controls receiving an equal volume of the alcoholic saline vehicle. There were no differences in the levels of circulating corticosterone between any of the groups. Further, neither acute nor chronic melatonin treatments had any demonstrable effect on plasma corticosterone concentrations either before or following ether stress. The increase in plasma corticosterone following ether did not differ between any of the groups. Melatonin injections did not differentially alter pituitary ACTH content. Therefore, there is no evidence from these results that melatonin administered chronically or acutely in the relatively large dose used has any effect on the functioning of the pituitary adrenal system. This study is specific to the intact animal; studies have not been done with pinealectomized animals kept under chronic light or dark conditions, or with sympathectomized animals.

It is important to note, however, that the pituitary ACTH content of both vehicle and melatonin chronically injected groups was only 50% that of the acutely treated animals, while plasma ACTH and adrenocortical secretion were apparently not differentially affected by such chronic treatments. Recent data suggests that chronic saline injections without 1% ethanol have the same effect[8] (S. Levine, R. Conner and J. Vernikos-Danellis, personal communication 1968).

There are at least two possible interpretations for this decrease in pituitary ACTH concentration in the chronically treated animals. The chronic stress of injections may lead to either an excess of release of ACTH over

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Treatment	No. rats	% body weight gain from day 1 to day 10	Anterior pituitary weight M ± S.E.	ACTH anterior pituitary mU/mg	Plasma mU/100 ml	Plasma corticosterone µg/100 ml (M ± S.E.) 1st sample	2nd sample
Melatonin × 10 days	10	8%	8.44 ± 0.57	5.9[a] (4.5–7.1)	1.48 (0.8–1.9)	15.53 ± 1.90	44.0 ± 1.75
Vehicle × 10 days	10	8%	9.40 ± 0.48	6.6 (4.9–8.7)	1.48 (1.0–1.7)	11.66 ± 1.20	46.60 ± 2.13
Melatonin × 1 day	10	7%	9.30 ± 0.25	15.1 (12.1–18.8)	1.5 (0.9–1.9)	14.51 ± 1.50	46.71 ± 1.34
Vehicle × 1 day	10	7%	8.96 ± 0.43	12.0 (8.9–15.1)	1.5 (0.8–1.9)	10.62 ± 2.41	45.56 ± 3.17

[a] 95% confidence limits given in parentheses.

synthesis or a decrease in pituitary ACTH stores. Regardless of the mechanism involved in the decrease in pituitary ACTH content, this decrease is not reflected in plasma ACTH, adrenocortical secretion, or the adrenocortical response to stress. Thus, the present findings provide further evidence that pituitary ACTH content can dramatically decrease without affecting the functional integrity of the pituitary adrenal system[9,10].

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[10] Supported by MH No. 13259, HD No. 02881, MH No. 10808 and ONR No. 102-715.

Zusammenfassung. Es wurde festgestellt, dass Melatonin, ein angebliches Hormon aus der Pinealis, keine spezifische Wirkung auf die Sekretion von ACTH aus dem Hypophysenvorderlappen hat.

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Hormonal Control of the Reproductive Diapause in the Grasshopper, *Oedipoda miniata*

LEVY[1] observed that in the grasshopper, *Oedipoda miniata*, a reproductive inactivity or 'adult diapause', lasting from May–October, occurs under field conditions in Israel. During this reproductive diapause, adults feed and are quite active, but neither mating nor egg-laying takes place. The effect of implanted active corpora allata on this reproductive diapause constituted the subject of the present study.

A stock of *O. miniata* was reared in the laboratory from hatching till moult to adult stage. As expected the adults obtained entered into reproductive diapause. At the age of 17–52 days after moult to adult stage, 3 groups, each composed of a male and a female batch (9–13 grasshoppers per batch), were set up. The following treatments were administered: (1) implantation of active corpora allata, (2) sham operation and (3) no operation (unoperated controls). The implanted corpora allata were obtained from sexually mature (22–34 days after moult to adult stage), male *Locusta migratoria migratorioides* donors; such glands were shown to be active when used for heterospecific implantations[2]. Two pairs of corpora allata were implanted through the neck membrane into each *O. miniata* receptor. Sham operated controls received pieces of mandibular muscles (obtained from the same donors). The unoperated controls were simply separated from the stock. Each batch was put into a 12 l Perspex cage. After a recovery period of 3 days methodical observations on the sexual behaviour were started and egg-laying was checked daily.

Implantation of active corpora allata led to intensive egg-laying in receptor females (Table). The first egg-pod was laid on the 10th and the last on the 21st day after implantation; from the 22nd day onwards no more egg-pods were laid (in the field egg-laying period lasts over 2 months[1]), although 5 females survived till the 39th day after implantation when they were killed and dissected. In 4 females resorption bodies[3] were found in place of the proximal oöcytes; the next oöcyte in the ovarioles was usually small, only 0.7–1.0 mm long. In the fifth female a few full sized eggs (about 5 mm long) were found in the oviduct, but otherwise the state of the ovarioles was similar to that observed in the others. It seems that while implantation of active corpora allata led to the termination of the reproductive diapause by inducing egg-laying, the implanted glands probably became gradually inactive.

No egg-laying occurred in the control females (Table). Most of these controls were dissected between the 33rd and 40th day after treatment. No noticeable differences were found between sham operated and unoperated females. The maximal length of the proximal oöcytes varied between 0.7 and 1.4 mm in different females, and no, or small quantities of yolk were seen in these oöcytes. These small oöcytes were frequently found in the process of resorption.

Implantations of active corpora allata led to prominent sexual behaviour in the male receptors. The intensity of sexual behaviour reached a maximum during the second and the third week of the observations, then it greatly decreased. The sexual behaviour of the receptor males was more intensive toward receptor than toward control females, but even in the latter case it was still prominent. These results indicate that implantation of active corpora allata led to the termination of the reproductive diapause by inducing intensive sexual behaviour. It seems, however, that the implanted glands became

Egg-laying in female receptors of *O. miniata* (following implantation of 2 pairs of active corpora allata obtained from sexually mature *Locusta migratoria migratorioides* males, as compared with controls

Treatment	No. of females used	No. of females surviving more than 5 days following treatment	No. of egg-pods laid
Receptors of active corpora allata	10	8[a]	15[b]
Sham operated controls	13	9	0
Unoperated controls	9	9	0

[a] Out of these 8 females one died on the 11th day, another one on the 18th day and a third one on the 27th day after implantation.
[b] All the egg-pods were laid between the 10th and the 21st day after implantation.

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