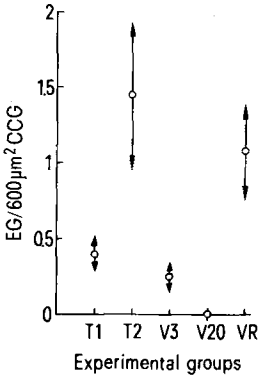
Number of EG per 600 μm² of CCG in different groups of locusts

T1	0.4 ± 0.12	(7)
T2	1.46 ± 0.49	(10)
V3	0.25 ± 0.10	(10)
V20	0.00 ± 0.00	(5)
VR	1.07 ± 0.32	(4)

Values are mean ± SD; number of determinations in parentheses. Experimental groups as for the figure.



Number of EG per 600 μm² CCG (mean ± SD) in resting 20-day adults (T1) and 40-day adults (T2), in 40-day adults having just flown for 3 h (V3) and 20 h (V20) and having flown at the age of 20 days for 20 h (VR).

(0.05 > p > 0.02). After a long flight (V20) no more EG were detectable, either in semi-thin or in ultra-thin sections. Finally if the long flight was followed by a resting period (VR), numerous and big EG were again observed ($p < 0.01$).

A good correlation was found between the presence of EG in the CCG and the flight activity of the locusts: in the controls many EG were observed; the number of EG decreased after a short flight and no EG could be detected after a long flight period.

Since the metabolic hormone of the CCG is released during flight[2-4], these results suggest the presence of an available hormonal content in the EG. In this case, the EG might serve for hormone storage. This would be in agreement a) with cytochemical data: EG and classical granules present similar cytochemical properties[6] and b) with chemical findings: the metabolic or adipokinetic hormone is a peptide[10,11] which can be entirely synthesized in the ergastoplasmic cisterns.

The EG, resulting in an asynchronism between the activities of the endoplasmic reticulum and the Golgi apparatus, may be a cytophysiological adaptation of the glandular cells of the CCG due to the heavy requirements occurring during the migration flights.

1 Research supported by grants A.T.P. No. 1831 and E.R.A. No. 620 from the Centre National de la Recherche Scientifique, French Ministry.
2 A.M.T. Beenakkers, J. Insect Physiol. *15*, 353 (1969).
3 R.J. Mayer and D.J. Candy, J. Insect Physiol. *15*, 611 (1969).
4 N.L. Robinson and G.J. Goldsworthy, J. Insect Physiol. *22*, 1559 (1969).
5 L.H.P. Rademakers and A.M.T. Beenakkers, Cell Tiss. Res. *180*, 155 (1977).
6 M. Lafon-Cazal, J. Micr. Biol. cell. *23*, 113 (1974).
7 M. Papillon, Bull. Biol. *93*, 204 (1960).
8 R. Michel, Rev. comp. Anat. *3*, 46 (1969).
9 R. Michel, F.O. Albrecht and D. Casanova, Acrida *5*, 299 (1976).
10 J.V. Stone, W. Mordue, K.E. Batley and H.R. Morris, Nature *263*, 207 (1976).
11 D.A. Holwerda, J. van Dorn and A.M.T. Beenakkers, Insect Biochem. *7*, 151 (1977).

Effect of an LH-RH antagonist on reproductive status of immature female rats

A. Corbin[1]

Endocrinology Section, Wyeth Laboratories Inc., Box 8299, Philadelphia (Pennsylvania 19101, USA), 4 November 1977

Summary. Administration of the LH-RH antagonist, D-Phe²-D-Ala⁶-LH-RH (Wy-18,185) to immature female rats from days 25–35 of age was without significant effect on day of vaginal opening (puberal onset), weights of the ovaries, uteri and anterior pituitary, and on ovarian histology on autopsy day 39.

Several investigations from this and other laboratories demonstrated the anti-luteinizing hormone (LH) and anti-ovulatory activity of a series of peptides derived from synthetic hypothalamic LH-releasing hormone (LH-RH)[2-5]. The antagonist, D-Phe²-D-Ala⁶-LH-RH (Wy-18,185), was of particular interest because of its extensive reproductive pharmacologic evaluation and reliability of anti-ovulatory activity[2]. Therefore, it was deemed of interest to determine if Wy-18,185 could impede the reproductive development of immature female rats.

Methods and materials. Immature, female Charles River CD® rats received a daily administration of 1.0 mg Wy-18,185, s.c. in corn oil, from days 25 through 35 of age. The occurrence of vaginal canalization was checked daily, and the animals were autopsied on day 39 of age (post-puberally). At autopsy, the weights of the body, thyroid, adrenal, uteri, ovaries and anterior pituitary gland were recorded. Statistical evaluation was performed using Student's t-test. The ovaries were fixed in 10% formalin, sectioned at 6 μm, stained with H and E and subjected to histologic evaluation.

Results and discussion. The data in the table demonstrate that chronic treatment of immature female rats with the LHRH antagonist was without any significant effect on the endocrine status of the recipients when autopsied post-puberally. Particularly, no dramatic effects were seen on the weights of the reproductive organs or on the day of vaginal opening (advent of puberty). Post-puberal histological evaluation of the ovaries of animals treated with the antagonist revealed normal ovarian structure, as evidenced by the presence of developing and antral follicles, ova, evidence of recent ovulation and luteinization.

Under the present experimental conditions, the antagonist was unable to effectively interfere with reproductive events in the prepuberal female, which is at variance with previous results derived from studies employing mature female animals[2-5]. However, it is possible that extended treatment and/or higher doses would have been effective, perhaps indicating that the immature female is less sensitive than the mature female to the inhibitory effects of the antagonists. This may, in turn, be a reflection of the different hypothalamic-hypophysial set-points that exist between im-

Endocrine status of immature female rats treated with Wy-18,185

Group	No. of rats	Body wt (g)	Thyroid wt (mg)	Adrenal wt (mg)	Uterine wt (mg)	Ovarian wt (mg)	Anterior pituitary wt (mg)	Day vagina opened
Oil control	20	128.6±2.6*	8.9±0.50	36.3±2.0	154.7±16	33.4±1.9	4.88±0.35	36.0±0.52
Wy-18,185	10	125.6±1.9	8.5±0.30	34.3±0.9	133.0±17	30.5±2.2	3.96±0.43	36.3±0.68

* Mean ± SE.

mature and adult female rats in relation to the complex interplay of peptide sensitivity and the steroid milieu[6], especially since Wy-18,185 has been shown to dually inhibit, by independent mechanisms, the release of hypothalamic LH-RH[7] and the release of pituitary LH[8]. Furthermore, Wy-18,185 and structurally related antagonists effectively block the pre-ovulatory gonadotropin surge and subsequent ovulation in the cyclic laboratory rodent, a rhythmic event that has yet to occur in the immature animal.

1 The technical expertise of J. Tracy is gratefully acknowleged.
2 A. Corbin and C.W. Beattie, Endocr. Res. Commun. *2*, 1 (1975).
3 C.W. Beattie, A. Corbin, T.J. Foell, V. Garsky, W.A. McKinley, R.W.A. Rees, D. Sarantakis and J.P. Yardley, J. med. Chem. *18*, 1247 (1975).
4 C.W. Beattie, A. Corbin, T.J. Foell, V. Garsky, R.W.A. Rees and J. Yardley, Contraception *13*, 341 (1976).
5 J.A. Vilchez-Martinez, D.H. Coy, E.J. Coy, A. Arimura and A.V. Schally, Endocr. Res. Commun. *3*, 231 (1976).
6 A. Corbin, in: Sexual Maturity: Physiological and Clinical Parameters, vol. 3, p. 37. Ed. E.S.E. Hafez and J.J. Peluso. Ann Arbor Sciences Publishers Inc., Ann Arbor 1976.
7 A. Corbin and C.W. Beattie, Endocrinology *98*, 247 (1976).
8 J.P. Yardley, T.J. Foell, C.W. Beattie and N.H. Grant, J. med. Chem. *18*, 1244 (1975).

The effects of microinjection of carbachol or hemicholinium into the amygdala on the levels of plasma and adrenal corticosterone in rats

A. Kawa, S. Ryu, Y. Taniguchi, K. Mizuguchi, Y. Maeda, T. Ariyama, K. Kamisaki, F. Koreeda and T. Kanehisa

The First Department of Internal Medicine, Kagoshima University Medical School, Kagoshima 890 (Japan),
4 November 1977

Summary. Microinjection of 0.4 µg of carbachol into the amygdala caused a rise of corticosterone (CS) in the morning, when the prestimulating level of CS was lower. But the same procedure with a larger dose had no effect in the afternoon, when the prestimulating level of CS was higher.

It is widely recognized that the regulation of the pituitary-adrenocortical (PA) functions requires the presence of elements in both the hypothalamic and the extrahypothalamic central nervous systems. Some conflicting evidence[1-6], however, regarding the role of the amygdala in the regulation of PA activities has been reported. This work deals with the effects of microinjection of carbachol (Carb) or hemicholinium (HC-3) into the amygdala on the levels of plasma and adrenal corticosterone (CS) in rats.

Materials and methods. Male Wistar rats weighing 300–380 g were used. Permanent cannuli, for microinjection of chemicals into the bilateral amygdala, were implanted stereotaxically. Microinjection was carried out 14 days after surgery. The doses were 0.1, 0.4 or 0.8 µg for Carb and 0.5, 1.0 or 5.0 µg for HC-3. The chemicals were dissolved in 0.5 µl of 0.9% saline and injected into the amygdala in a volume of 0.5 µl for each site. The same quantity of the vehicle was given to the control animals. Then experiments were carried out at 08.00 and 15.00 h. The animals were killed 40 min after microinjection of the chemicals. Trunk blood was collected and centrifuged. The right adrenal gland was removed, weighed, homogenized in saline containing 20% ethanol. The samples were kept frozen until assayed fluorometrically for their CS content, according to

Effects of microinjection of carbachol or hemicholinium into the amygdala on basal levels of plasma and adrenal corticosterone in rats

	n	Plasma corticosterone (µg/100 ml)	Adrenal corticosterone (µg/100 mg)
Morning			
Control	7	5.99± 1.57	0.12±0.12
Carb (0.4 µg)	7	19.94±15.26*	1.41±2.18
Control	7	5.31± 1.78	0.03±0.03
HC-3 (1.0 µg)	8	5.99± 2.53	0.02±0.04
Afternoon			
Control	8	21.71± 9.98	1.15±0.74
Carb (µg)			
0.1	8	20.91±10.13	1.01±0.43
0.4	8	21.66±11.30	0.80±1.04
0.8	8	23.30±15.35	1.54±1.37
Control	8	16.50± 8.46	0.92±1.20
HC-3 (µg)			
0.5	7	21.11±11.11	1.79±1.70
1.0	8	14.27± 5.60	0.86±1.31
5.0	7	22.11±10.02	1.90±1.40

Control animals were injected with saline. Values are expressed as mean ± SD. n, refers to the number of animals used; *: $p < 0.05$ as compared with controls; Student's t-test.