Table I. Effect of Methallibure on the testis of *R. cyanophlyctis*

Group	Average testis Wt. (mg/100 g body Wt. ± SE)	Average diameter (μm ± SE)			Δ^5-β HSDH activity[a]	G-6-PDH activity[a]
		Testis	Testis-tubule	Leydig cell nucleus		
Control	260.94 ± 34.7	1858.5 ± 25.2	251.7 ± 10.18	4.48 ± 0.01	++	++++
Experimental	249.76 ± 25.37 $p > 0.5$	1748.2 ± 77.8 $p < 0.3$	235.7 ± 4.44 $p < 0.3$	4.05 ± 0.06 $p < 0.001$	+	++

SE, Standard error. [a] Intensity of reaction is visually graded from (+) to (++++); p-values calculated by Student's t-test between control and experimental groups.

Table II. Effect of Methallibure on the testis cholesterol in *R. cyanophlyctis*

	μg/100 mg wet weight of testis		Increase over control value (%)
	Control	Experimental	
Free cholesterol	440.43	447.10	1.51
Total cholesterol	559.28	763.00	18.54

Table III. Effect of Methallibure on the thumb pad of *R. cyanophlyctis*

Group	Average height μm ± SE	
	Epidermis	Glandular epithelium
Control	78.05 ± 7.77	24.38 ± 2.76
Experimental	63.55 ± 9.00 $p < 0.4$	11.93 ± 0.64 $p < 0.01$

SE, standard error. p-values calculated by Student's t-test between control and experimental groups.

glandular epithelium of thumb pads decreased markedly (Table III) in the treated specimens. The epidermis was less papillate and the mucous glands were atrophic in the treated specimens. These observations suggest an impaired androgen production by the testes which is reflected in the regression of the thumb pad, an androgen dependent secondary sexual character. The present findings are in conformity with those reported earlier on other species[1-5] wherein Methallibure was found to cause regression of the secondary sex characters in male. However, the low dose of Methallibure used did not significantly influence the testicular histology and histometry during the short-term treatment.

Zusammenfassung. Die Behandlung des männlichen Frosches *Rana cyanophlyctis* mit Methallibur (ICI Verbindung 33, 828) während der Dauer von vier Wochen ergab die folgenden Wirkungen: 1. Rückbildung der Leydigschen Zellen und Abnahme ihrer Δ^5-3β-HSDH-Aktivität, 2. Anstieg des Totalgehaltes an Cholesterol, und 3. Rückbildung der Daumenschwielen. Bedeutende histologische und histochemische Veränderungen der Samenkanälchen wurden jedoch nicht beobachtet.

S. K. Saidapur[9], S. R. Ganihar and V. B. Nadkarni[10,11]

Department of Zoology, Karnatak University, Dharwar 580 003 (India), 16 December 1974.

[9] Supported by a grant No. 4756 from the U.G.C. of India.
[10] Supported by a grant No. M66.117 from the Population Council, Bio Medical Division, New York.
[11] We are grateful to Imperial Chemical Industries Limited, England, for the free gift of Methallibure and to Dr. M. A. Rao for providing laboratory facilities.

Effect of Pimozide, a Dopaminergic Blocking Agent, on Hypothalamic Luteinizing Hormone Releasing Hormone Activity in Hypophysectomized Rats

Hypothalamic releasing factor (RF) mechanisms appear to be regulated by diencephalic dopaminergic systems[1,2]. RF activity has been detected in the plasma of hypophysectomized animals[3,4], thus providing a model in which the effects of pharmacologic agents on the hypothalamic neurotransmitter/RF function can be evaluated. In view of our recent report[4] describing the elimination of plasma luteinizing hormone (LH) releasing hormone (LRF) activity in hypophysectomized rats with the

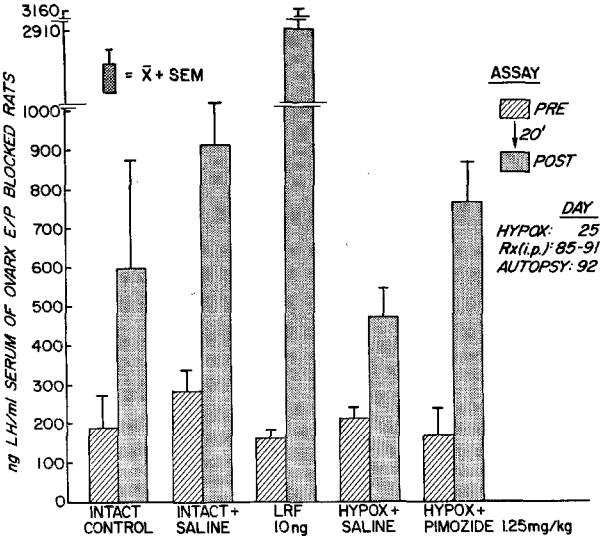
[1] T. Hökfelt and K. Fuxe, in *Brain-Endocrine Interaction* (Eds. K. M. Knigge, D. E. Scott and A. Weindl; S. Karger, Basel 1972), p. 181.
[2] S. M. McCann, P. S. Kalra, A. O. Donoso, W. Bishop, H. P. G. Schneider, C. P. Fawcett and L. Krulich, in *Brain-Endocrine Interaction* (Eds. K. M. Knigge, D. E. Scott and A. Weindl; S. Karger, Basel 1972), p. 224.
[3] A. Corbin, E. L. Daniels and J. E. Milmore, Endocrinology *86*, 735 (1970).
[4] A. Corbin and G. V. Upton, Experientia *29*, 1552 (1973).

dopaminergic blocking agent pimozide, it was deemed important to investigate hypothalamic LRF activity as well.

Methods and materials. Female Sprague-Dawley (S-D) rats were hypophysectomized when 25 days old and used 60 days later. Rats (10–15/group) received pimozide[5] i.p. from days 85–91. On the day after the last injection the rats were sacrificed and the stalk-median eminences (SME) obtained for LRF assay. SME's also were obtained from intact female rats of the same age.

The SME's were homogenized in 0.1 *N* HCl, centrifuged (3000 rpm/20 min), and the supernatant material was immersed in a boiling water bath for 10 min and then diluted with the acid to a concentration corresponding to 2 SME/ml.

The LRF activity of the crude SME extracts was evaluated by measuring the amount of LH released into the serum of adult ovariectomized (OVARX) S-D rats that had been treated 72 h earlier with s.c. doses of 50 µg estradiol benzoate (E) plus 25 mg progesterone (P) (5 rats/group). A blood sample was obtained before and 20 min after i.v. injection of 2.0 SME/ml donor tissue into each assay rat. Serum LH levels were determined by the double antibody radioimmunoassay technique of NISWENDER et al.[6] and are expressed in terms of NIAMD-Rat LH-RP-1. Levels of significance were calculated on basis of % change in serum LH.



Effect of pimozide on SME-LRF activity of hypophysectomized female rats.

Results and discussion. The data are presented in the Figure. The responsiveness of the OVARX E/P assay rats was established by injection of 10 ng of synthetic LRF[7], which resulted in a very large and significant ($p \leq 0.01$) rise in serum LH. SME's derived from hypophysectomized, saline treated rats possessed less LRF activity than that found in SME tissue obtained from respective intact controls ($p \leq 0.05$). Treatment of hypophysectomized rats with 1.25 mg/kg pimozide produced a significant rise in SME-LRF activity when compared to the hypophysectomized saline treated group ($p \leq 0.02$).

The collective data derived from our laboratory[4] reveal the following: intact female rats possess SME-LRF activity that is significantly higher than that of hypophysectomized rats. In contrast to the high plasma LRF activity of hypophysectomized animals, no such activity could be found in the peripheral circulation of the intact rats[4]. Treatment of hypophysectomized rats with the central nervous system dopamine receptor blocker and neuroleptic[8,9], pimozide, eliminates plasma LRF activity[4], associated with a rise of LRF activity in the SME. These data indicate, at the least, that pimozide can inhibit the release of hypothalamic LRF.

The concept that hypothalamic dopaminergic systems subserve hypophysiotropic area RF function has received considerable support[10,11]. The recent study of UPTON and CORBIN[12] demonstrated that administration of pimozide to hypophysectomized and intact rats produces an increase in corticotropin releasing factor (CRF) activity in the SME. The assembled data suggest that dopaminergic mechanisms are involved in the control of the release of hypothalamic RF's[13].

Résumé. L'administration de pimozide, un bloqueur du récepteur dopaminergique, provoque chez la ratte hypophysectomisée une élévation marquée de l'activité LRF de l'éminence médiane. Les résultats suggèrent que la libération des «releasing hormones» hypothalamiques est réglée par les mécanismes dopaminergiques.

A. CORBIN, C. W. BEATTIE and G. VIRGINIA UPTON

Endocrinology Section, Wyeth Laboratories, Research Division, Box 8299, and Wyeth International Ltd., Philadelphia (Pennsylvania 19101, USA), 2 December 1974.

[5] Janssen Pharmaceutica, Beerse, Belgium.

[6] G. D. NISWENDER, A. R. MIDGLEY, JR., S. E. MONROE and L. E. REICHERT, JR., Proc. Soc. exp. Biol. Med. *128*, 807 (1968).

[7] Wyeth Compound No. 16,558.

[8] P. JANSSEN, J. BRUGMANS, J. DONY and V. SCHUERMANS, J. clin. Pharmac. *12*, 26 (1972).

[9] N. E. ANDEN, S. G. BUTCHER, H. CORRODI, K. FUXE and U. UNGERSTEDT, Eur. J. Pharmac. *11*, 303 (1970).

[10] R. I. WEINER, R. A. GORSKI and C. H. SAWYER, in *Brain-Endocrine Interaction* (Eds. K. M. KNIGGE, D. E. SCOTT and A. WEINDL; S. Karger, Basel 1972), p. 236.

[11] J. C. PORTER, I. A. KAMBERI and J. G. ONDO, in *Brain-Endocrine Interaction* (Eds. K. M. KNIGGE, D. E. SCOTT and A. WEINDL; S. Karger, Basel 1972), p. 224.

[12] G. V. UPTON and A. CORBIN, Int. Symp. Hypothalamic Hormones, Milano, October 1974.

[13] The technical assistance of J. BELL, G. COLE, K. KOCH, P. MARTIN and J. Tracy is gratefully acknowledged.