

## Effect of pinealectomy and 5-methoxytryptophol administration on the gonads and accessory sex organs of male Indian palm squirrel, *Funambulus pennanti*

N. SAXENA, E. A. KHAN, M. P. SINHA and P. N. MEHROTRA  
Post Graduate Department of Zoology, Ranchi University, Ranchi - 834 008.

**Abstract.** Subcutaneous evening injections of 5-methoxy-tryptophol (ML), for forty five continuous days, resulted in a significant decrease in the testis and epididymal weight of sexually active pinealectomized and intact *Funambulus pennanti*. A significant reduction was also noted in the glycerylphosphoryl choline content of epididymis. ML injections failed to affect the seminal vesicle and prostate gland weight and activity. It is concluded that ML inhibits gonadal activity and is a biologically active pineal compound in *F. pennanti*.

### Introduction

The seasonal change in reproductive activity is controlled by the pineal gland in most species of animals. The indole derivatives of the gland play a major role in this process. It is now evident that besides melatonin, the pineal gland synthesizes four other different 5-methoxyindoles; 5-methoxytryptamine, 5-methoxytryptophan, 5-methoxyindole-acetic acid and 5-methoxytryptophol (ML) all of which are potentially active factors (Pevet, 1983; Pevet 1985 a, b). Most of the research on pineal gland has centred on the role of melatonin in pineal function (Stetson and Watson-Whitmyre, 1984). Studies related to pineal control of reproduction involving other 5-methoxyindoles are scanty in Indian tropical animals. Therefore, the aim of the present investigation has been to observe the effect of ML injections on the gonads and accessory sex organs of a tropical mammal, *Funambulus pennanti*. The study was also performed in pinealectomized (Px) animals in order to test the pineal dependency/independency of this 5-methoxyindole, in affecting gonadal activity.

### Materials and Methods

The study was performed during June-July which coincided with the sexually active phase of the annual testicular cycle of the animal. 32 adult (100-120 g body weight) males of *F. pennanti* were obtained in the first week of June and acclimatized to laboratory conditions for 2 weeks. They were housed in wire net cages and had an easy access to food and water *ad libitum*. The animals were divided randomly into the following four groups of eight animals each :

Group I : The animals were Px and injected with 10  $\mu$ g ML/animal/day.

Group II : The animals were sham-operated (SO) and injected with 10  $\mu$ g ML/animal) day.

Group III: The animals were Px and injected with 0.1 ml vehicle (normal saline, 0.9% NaCl) animal/day.

Group IV: The animals were SO and injected with 0.1 ml vehicle/animal/day.

All injections were given subcutaneously between 1650-1700 hrs. Px was performed following the method of Haldar-Misra (1986). ML was obtained from Sigma Chemical Co., U. S. A. Groups III and IV served as controls for Groups I and II respectively.

After completion of 45 days, the animals were sacrificed by cervical dislocation and their body weights noted. Testes and accessory sex organs were removed and weighed on a microelectrical balance. Biochemical estimations of glycerylphosphoryl choline (GPC) content of epididymis, fructose level of seminal vesicle and acid phosphatase activity of prostate gland were done by the methods of White (1959), Mann (1946) and Varley (1967) respectively. The data was analysed statistically by Student's *t* test (Bruning and Kintz, 1977).

### Results

ML administration significantly reduced the testes weight of SO as well as Px animals. A significant decrease was also observed in the epididymis weight and its GPC content of animals injected with ML (Tables 1 & 2). ML injections were without any effect on the seminal vesicle and prostate gland weights and their respective fructose level and acid phosphatase activity in both SO and Px squirrels (Tables 1 & 2).

**Table 1.** Effect of evening injections of 5-methoxytryptophol (ML) on the testis and accessory sex organ weight (g/100 g body weight) of pinealectomized (Px) and sham-operated (SO) *F. pennanti*, during the active phase of the annual gonadal cycle.

	Testis	Epididymis	Seminal vesicle	Prostate gland
Px Control	0.89 ± 0.005	0.26 ± 0.005	0.16 ± 0.004	0.18 ± 0.005
SO Control	0.83 ± 0.01	0.20 ± 0.004	0.16 ± 0.002	0.18 ± 0.01
Px + ML	0.79 ± 0.004†	0.19 ± 0.006†	0.16 ± 0.005	0.19 ± 0.02
SO + ML	0.78 ± 0.004†	0.14 ± 0.004†	0.15 ± 0.006	0.17 ± 0.01

Significance of difference from control: †,  $p < 0.001$ .

**Table 2.** Effect of evening injections of 5-methoxytryptophol (ML) on the fructose level (seminal vesicle), acid phosphatase activity (prostate gland) and glycerylphosphoryl choline content (epididymis) of pinealectomized (Px) and sham-operated (SO) *F. pennanti*, during the active phase of the annual gonadal cycle.

	Fructose ( $\mu\text{g}/100$ mg tissue)	Acid phosphatase (K.A. units/100 mg tissue)	Glycerylphosphoryl choline ( $\mu\text{g}/100$ mg tissue)
Px Control	312.75 ± 11.98	54.00 ± 4.90	68.87 ± 1.12
SO Control	311.12 ± 14.29	48.75 ± 3.26	57.87 ± 3.90
Px + ML	280.87 ± 18.79	49.62 ± 5.09	52.12 ± 1.75††
SO + ML	284.00 ± 16.14	45.87 ± 4.30	42.12 ± 1.00†

Significance of difference from control: †,  $p < 0.005$ ; ††,  $p < 0.001$ .

### Discussion

The results of the present study clearly indicate that exogenous ML can inhibit the gonads of *F. pennanti* independently of the pineal gland. The study also shows that Px stimulates the testis of the squirrel. Administration for 45 days is sufficient for the expression of this inhibitory effect of ML. While considering the accessory sex organs it was observed that although ML inhibited the epididymal activity, as evident by the lowered GPC content of injected animals, it was unable to affect the other two accessory sex organs. Seminal vesicle and prostate gland failed to show any response to ML administration. It is suggested that perhaps Px has a differential effect on the gonads and accessory sex organs of *F. pennanti* or Px may be interfering differently with the androgen receptor activity of these two organs. This has also been suggested,

in earlier studies, for similar results of pineal removal on the accessory sex organs of *F. pennanti* (Haldar and Saxena, 1990).

Literature reveals that ML, like melatonin and 5-methoxytryptamine, is capable of modifying sexual development in birds and mammals (Balemans, 1972; Balemans *et al.*, 1977a, b). In juvenile chicken it stimulates gonadal growth whereas in mature and adult birds it exhibits an inhibitory effect. In mouse, it is reported to inhibit ovarian and uterine hypertrophy induced by injections of human chorionic gonadotrophin or pregnant mare serum gonadotrophin (Vaughan *et al.*, 1976). The present study clearly demonstrates an antigonadotrophic effect in case of *F. pennanti*. Responses of Px and intact squirrels indicate that the effects of exogenous ML are not mediated via the pineal gland. Thus, ML appears to be involved in control of reproductive activity of *F. pennanti*. Its precise physiological properties need further investigation.

#### Acknowledgements

This study was supported by a grant, to the first author, from CSIR, New Delhi.

#### References

- Balemans, M. G. M. 1972. Age dependent effects of 5-methoxytryptophol and melatonin on testes and comb growth of the White Leghorn *Gallus domesticus*, L.). *J. Neural Transm.*, **33**, 179-194.
- Balemans, M. G. M., Van der Veerdonk, F. C. G. and Van de Kamer, J. C. 1977a. Effect of injecting 5-methoxyindoles on testicular weight of White Leghorn cockerels. *J. Neural Transm.*, **41**, 47-54.
- Balemans, M. G. M., Van der Veerdonk, F. C. G. and Van de Kamer, J. C. 1977b. The influence of 5-methoxytryptophol, a pineal compound, on comb growth, ovarian weight, follicular growth and egg production of juvenile and maturing adult White Leghorn hens. *J. Neural Transm.*, **41**, 37-46.
- Bruning, J. L. and Kintz, B. L. 1977. In: *Computational Handbook of Statistics* (ed) J. L. Bruning and B. L. Kintz, Scott Foresman and Co. Palo, Alto, Calif.
- Haldar-Misra, C. 1986. Methods of pinealectomy in some vertebrates. *Indian J. Exp. Biol.*, **24**, 319-322.
- Haldar, C. and Saxena, N. 1990. Ecofactors and effect of pinealectomy in regulation of seasonal testicular cycle in the Indian palm squirrel, *Funambulus pennanti*. *J. Interdiscipl. Cycle Res.*, **21**, 1-11.
- Mann, T. 1946. Colorimetric method for the estimation of fructose. *Biochem. J.*, **40**, 481.
- Pevet, P. 1983. The 5-methoxyindoles different from melatonin: Their effect on the sexual axis. In: *The Pineal Gland and its Endocrine Role* (eds, J. Axelrod, F. Fraschini and G. Velo), Plenum Publishing Corporation, New York and London, p. 331.
- Pevet, P. 1985a. 5-Methoxyindoles, pineal and seasonal reproduction. A new approach. In: *The Pineal Gland, Current State of Pineal Research* (eds, B. Mess, C. S. Ruzsas, L. Tima and P. Pevet), Elsevier Sciences Publisher, Amsterdam, p. 163-186.
- Pevet, P. 1985b. 5-Methoxyindoles, Pineal Peptides and Reproduction. In: *The Pineal Gland Endocrine Aspects* (eds. G. M. Irown and S. B. Wainright), Pergamon Press, Oxford, pp. 81-102.
- Stetson, M. H. and Watson-Whitmyre 1984. Physiology of the pineal and its hormone malatonin in annual reproduction in rodents. In: *The Pineal Gland* (ed. R. J. Reiter), Raven Press, New York, pp. 109-154.
- Varley, H. 1967. In: *Practical Clinical Biochemistry* (ed. H. Varley), William Heinemann Medical Books Ltd., London, P. 46.
- Vaughan, M. K., Vaughan, M. G. and Reiter, R. J. 1976. Inhibition of human chorionic gonadotrophin induced hypertrophy of the ovaries and uterus of immature mice by some pineal indoles, 6-hydroxy-melatonin and arginine vasotocin. *J. Endocrinol.*, **68**, 397-400.
- White, I. G. 1959. Studies on the estimation of glycerol, fructose and lactic acid with particular reference to semen. *Aus. J. Exp. Biol. Med.*, **37**, 441-450.