Effect of orally administered melatonin on reproductive function of the golden hamster

P. Pévet[1] and C. Haldar-Misra

The Netherlands Institute for Brain Research, IJdijk 28, NL-1095 KJ Amsterdam (The Netherlands), 5 March 1982

Summary. Melatonin, given in the drinking-water, induces gonadal atrophy in the male golden hamster maintained in a light/dark cycle (14:10 h).

It is increasingly evident that the pineal gland is principally involved in long-term adaptation of functions such as reproduction to environmental conditions, especially in seasonal breeders. Although there is still controversy on the classification of melatonin (MEL) as a pineal hormone, there is no doubt that this compound is deeply inplicated in this phenomenon of adaptation[2-6]. The action of MEL, however, appears to be very complex. It can, for example, either inhibit or promote gonadal growth and function, depending on its mode of administration (injections, s.c. deposits) and the time of administration. Recently, Kennaway and Seamark[7] have observed in ruminants (goat and sheep) that oral administration of MEL either in saline solution or absorbed into pelleted food, resulted in a sustained elevated blood level for periods exceeding 7 h. This observation permits us to conclude that, at least in ruminants, the oral route of administration provides a convenient way of administering MEL for physiological study.

The biological effects of MEL have been extensively studied in the hamster, in which clear results such as gonadal atrophy simply measured by weight of the testis have been obtained[2,3]. It therefore seemed to be of importance to us to look at the effect of orally administered MEL on the reproductive axis in the hamster.

Materials and methods. All animals used in this study were young adult male golden hamsters *(Mesocricetus auratus)* (60–70 g at the onset of the study) obtained from TNO, Zeist, The Netherlands, where they had been raised at a photoperiodic schedule of LD 14:10 h. After 2 weeks acclimatization (always in 14:10) the animals were divided into 4 groups of 5 animals each. Two of these groups (I and II) were kept under short-day conditions (LD 10:14 h i.e. light from 08.00 to 18.00 h) and the 2 other groups (III and IV) under long-day conditions (14:10, i.e. light from 04.00

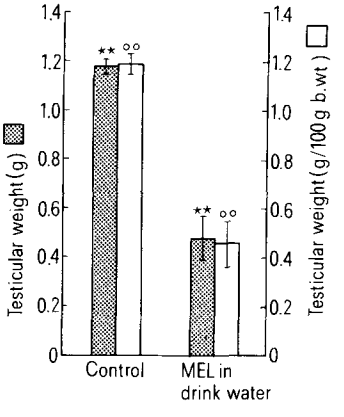

Figure 1. Mean absolute (dotted bars) and relative (empty bars) testicular weight of hamsters kept under long photoperiod after melatonin treatment. Group III, control; group IV, MEL in drinking-water. Vertical lines at top of the bars signify SE. **, °°$p < 0.01$.

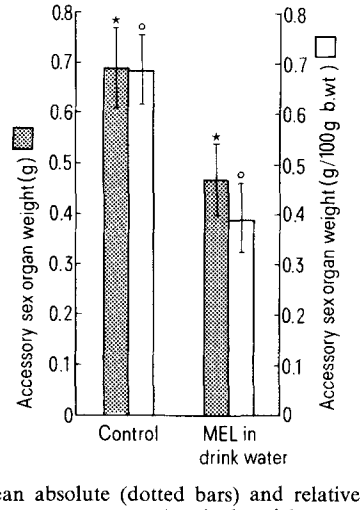

Figure 2. Mean absolute (dotted bars) and relative (empty bars) weight of accessory sex organ (seminal vesicles, coagulating gland and secretion content) of hamsters kept under long photoperiod after melatonin treatment. Group III, control; group IV, MEL in drinking-water. *, °$p < 0.05$.

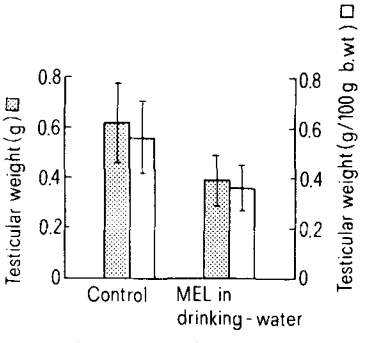

Figure 3. Mean absolute (dotted bars) and relative (empty bars) testicular weight of hamsters kept under short photoperiod after melatonin treatment. Group I, control; group II, MEL in drinking-water.

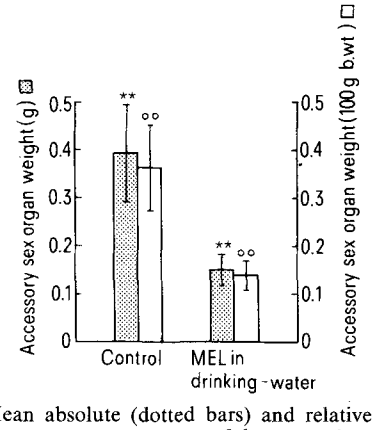

Figure 4. Mean absolute (dotted bars) and relative (empty bars) weights of accessory sex organs of hamsters kept under short photoperiod after melatonin treatment. Group I, control; group II, MEL in drinking-water. **, °°$p < 0.01$.

to 18.00 h). All animals received tap water and food ad libitum. Groups II and IV, however, had MEL dissolved in the drinking-water. Based on preliminary observations of the quantity of water drunk by the animals we calculated that in order to obtain a MEL ingestion of approximately 50 µg/day/hamster, we had to use a concentration of 2.5 mg/500 ml water. The 2.5 mg MEL were dissolved in 200 µl ethanol which were then mixed with 500 ml water. MEL-containing drinking-water was refreshed every week. Ethanol (200 µl/500 ml tap water) was added to the drinking-water of the control groups (I, III). After 8 weeks the hamsters were sacrificed and the testes and accessory sex organs (seminal vesicles and coagulating glands) were dissected and weighed. The results were statistically analyzed using Student's t-test and are given as means ± SEM.

Results and discussion. As shown in figures 1 and 2, MEL orally administered via the drinking water caused a marked loss of testicle and accesory sex organ weight in hamsters maintained in long photoperiod, suggesting a strong antigonadotropic effect. Short photoperiod by itself is known to induce gonadal atrophy in hamsters[2,3] and this is visible when we compare the control groups submitted to short days (figs 3 and 4) with control groups submitted to long days (figs 1 and 2). MEL does not seem to have any effect in hamsters maintained in short photoperiod, at least when the testes are considered (fig. 3). Looking at the sex organs, however, it appears that orally administered MEL could also have an inhibitory effect. The weight of the accessory organs of the MEL-treated hamsters (fig. 4) is, indeed, significantly lower than in the control group. This result seems to suggest that MEL could also have an antigonadal effect in hamsters kept in short photoperiod, an effect which would be masked by the gonadal atrophy induced by the short photoperiod. If this interpretation is correct, such a result could be of great importance for the understanding of pineal physiology.

Kennaway and Seamark[7] have observed that s.c. injections of MEL resulted in an extremely rapid rise in plasma hormone titres to peak within 15 min, with an apparent half life for MEL of about 30 min. It appears thus that after injection, MEL disappears quickly, and indeed these authors have observed that after 4 h no MEL was detectable in the blood. Oral administration, on the contrary, resulted in blood MEL levels which rose within 30 min to a plateau which was sustained for at least 7 h. It is difficult at present to extrapolate these results obtained in ruminants to the hamster, but in our opinion it seems sound to assume that orally administered MEL induces an increase in blood MEL concentration for a longer time than does s.c. injection of MEL. How, therefore, can we integrate our observation that orally administered MEL induced gonadal atrophy in the hamster with the observations of other authors that in this species MEL is progonadotropic (or counterantigonadotropic) when constantly available, and antigonadotropic when injected late in the afternoon[2,3]? S.c. injections of MEL, however, induced gonadal atrophy not only when injected in the late afternoon but also when injected at the beginning or at the end of the dark period[8]. The hamster is a nocturnal animal. This means that it is active during the dark period, especially at the beginning and end of it, and that consequently it eats and drinks at these times. This probably explains why orally administered MEL in the hamster has an antigonadotropic effect. On the other hand, as it has been demonstrated that multiple daily injections of MEL induced gonadal atrophy in hamster[9,10], and as the animals probably drink more than once a day, it could be possible that it is this multiple daily ingestion of MEL which is responsible for the observed antigonadotropic effect.

In conclusion, it appears that the inhibition of gonadal function normally observed after multiple daily injections, or late afternoon injections, can also be obtained by simply adding MEL to the drinking-water. For future studies, MEL orally administered via the drinking-water seems to be a very effective and especially a very practical way of administration.

1 Acknowledgments. The authors wish to thank Ms E. de Graaf for her skillful technical assistance.
2 Reiter, R.J., Endocrine Rev. *1* (1980) 109.
3 Hoffmann, K., in: The pineal organ: photobiology, biochronometry, endocrinology, p. 123. Eds A. Oksche and P. Pévet. Elsevier, Amsterdam 1981.
4 Pévet, P., Thesis, University of Amsterdam, Amsterdam 1976.
5 Pévet, P., Haldar-Misra, C., and Öcal, T., J. neural Transm. *52* (1981) 95.
6 Pévet, P., Haldar-Misra, C., and Öcal, T., J. neural Transm. *51* (1981) 303.
7 Kennaway, D.J., and Seamark, R.F., Aust. J. biol. Sci. *33* (1980) 349.
8 Tamarkin, L., Lefebvre, N.G., Hollister, C.W., and Goldman, B.D., Endocrinology *101* (1977) 631.
9 Tamarkin, L., Hollister, C.W., Lefebvre, N.G., and Goldman, B.D., Science *198* (1977) 935.
10 Goldman, B., Hall, V., Hollister, C.W., Roychoudhury, P., Tamarkin, L., and Westrom, W., Endocrinology *104* (1979) 82.

The effect of o,p'-DDD on the adrenal cortex in sheep[1]

R. Thun[2], P. Wild, F. Mettler and M. Djafarian

Institut für Zuchthygiene, and Veterinär-Anatomisches Institut, Universität Zürich, Winterthurerstrasse 260, CH-8057 Zürich (Switzerland), and Bristol-Myers SA, CH-6330 Cham (Switzerland), 6 Januray 1982

Summary. The effect of o,p'-DDD (200 mg/kg/day given p.o. for 100 consecutive days) on the sheep adrenal gland was studied. The results suggest that this ruminant species is highly resistant to the adrenocorticolytic activity of o,p'-DDD when compared with dogs.

The compound 1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl) ethane, known by its trivial name o,p'-DDD, was shown first in the dog to be selectively toxic to the adrenal cortex[3,4]. Histologic and ultrastructural studies in dogs[5,6] suggest a direct toxic effect on mitochondria, resulting in cellular necrosis and atrophy of the adrenal gland. Cytotoxicity is most pronounced in the zona fasciculata and zona reticularis whereas the zona glomerulosa is only mildly affected. In addition, o,p'-DDD has been shown to block ACTH-induced steroidogenesis[7] and to modify the peripheral metabolism of steroids[8]. Today, o,p'-DDD is widely used for the treatment of adrenal cortical neoplasia and hyperadrenocorticism in man[9,10] and in animals[11]. In order to extend our studies about the endocrine relationship