stance mucopolysaccharides and collagen and in changes in osmotic pressure that follow². Similarly, vascular permeability may be modified by depolymerization and dissagregation of basement membrane collagen fibrils in small uterine blood vessels, noted following estrogen treatment, and by released prostaglandins². Histamine release from uterine mast cells, in turn, may be influenced by released prostaglandins²⁰ and/or the basic protein fraction liberated from eosinophils, in a similar way to that described for peritoneal mast cells²¹.

- Acknowledgments. This work was supported by the Belgian Ministry of Scientific Policy (Concerted Actions of Research) and by the Servicio de Desarrollo Científico, Artistico y de Cooperacion Internacional from the University of Chile.
- A. Tchernitchin, J. Steroid Biochem. 11, 417 (1979).
- R.L. Singhal and R.T. Lafreniere, J. Pharmac. exp. Ther. 180, 86 (1972).
- A. Tchernitchin, X. Tchernitchin, A. Rodríguez, M.A. Mena, C. Unda, N. Mairesse and P. Galand, Experientia 33, 1536
- E.V. Jensen and E.R. DeSombre, A. Rev. Biochem. 41, 203 (1972).
- A. Tchernitchin and X. Tchernitchin, Experientia 32, 1240 (1976).

- B.M. Lippe and C.M. Szego, Nature 207, 272 (1965).
- A. Tchernitchin, X. Tchernitchin and P. Galand, Differentiation 5, 145 (1976).
- K. Burton, in: Methods in Enzymology, vol. 12, p. 163. Eds. L. Grossman and K. Moldave. Academic Press, New York 1968.
- Z. Dische, in: The Nucleic Acids, vol.1, p.301. Eds H. Chargaff and J.N. Davidson. Academic Press, New York 1955.
- O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. 193, 265 (1951).
- R. Montgomery, Archs Biochem. 67, 378 (1957). V.V. Rogovin, V.M. Frolova, N.G. Geranina, R.A. Muravieff and L.A. Piruzyan, Izv. Akad. Nauk SSSR, Ser. Biol. 1, 144
- J.R. Baker, P.A. Baker, E.G. Bassett and D.B. Myers, J. Physiol., Lond. 263, 242P (1976).
- G.T. Archer and J.G. Hirsch, J. exp. Med. 118, 277 (1963).
- R.R. Vercauteren and G. Peeters, Archs int. Pharmacodyn. Ther. 89, 10 (1952).
- 17 I. Olsson, P. Venge, J.K. Spitznagel and R.I. Lehrer, Lab. Invest. 36, 493 (1977)
- T. Hubscher, J. Immun. 114, 1379 (1975).
- A. Tchernitchin, J. Steroid Biochem. 4, 277 (1973).
- 20 A. Tauber, M. Kaliner, D. J. Stechschulte and K. F. Austen, J. Immun. 111, 27 (1973).
- J. Padawer, in: Modern Concepts and Developments in Immediate Hypersensibility, vol.7, p.301. Ed. M.K. Bach. Marcel Dekker, New York 1978.

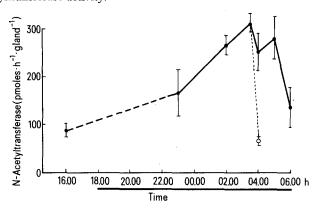
Effect of light at night on the pineal rhythm in N-acetyltransferase activity in the Syrian hamster Mesocricetus auratus

J. Vaněček¹ and Helena Illnerová

Institute of Physiology, Czechoslovak Academy of Sciences, Videnská 1083, 142 20 Prague 4 (Czechoslovakia), 4 May 1981

Summary. Pineal N-acetyltransferase activity in the male Syrian hamster exhibited a daily rhythm; the maximal night-time value was 3.5-fold higher than the day-time value. When hamsters were exposed to light at night N-acetyltransferase declined within 30 min to ½ of its former activity. These results indicate that in the Syrian hamster the pineal melatonin rhythm may be regulated at least partly via changes in N-acetyltransferase activity.

The enzyme N-acetyltransferase (acetyl-CoA arylamine N-acetyltransferase, EC 2.3.1.5) (NAT) produces the melatonin precursor N-acetylserotonin². It is supposed that the daily rhythm in the melatonin content in the rat pineal gland is driven by rhythmic changes in NAT activity³, as both NAT activity and melatonin concentration change in the same way throughout the day4 and respond similarly to light at night⁵⁻⁸. In Djungarian hamsters the melatonin rhythm is probably also driven by the rhythm in NAT activity. The ratio of night to day pineal NAT activity^{9,10}, as well as the ratio of night to day pineal concentration¹¹ is about 30-50. After exposure to light at night, both NAT activity and melatonin content decline rapidly. Different relations between NAT and melatonin may exist in Syrian hamsters. Though the ratio of night to day pineal melatonin content is about 10-20 in Syrian hamsters^{12,13}, Tamarkin et al.¹³ did not find any difference between day and night NAT activity. Moreover, the rapid decline in melatonin concentration in response to light at night was not accompanied by any decrease in NAT activity¹³. These observations led Tamarkin et al. 13 to the supposition that the regulation of changes in melatonin content in Syrian hamsters may not be affected by changes in pineal NAT activity. To find out whether a real difference exists in the regulation of pineal melatonin content between this species and other nocturnal rodents such as rats and Djungarian hamsters, we studied the NAT rhythm and the response of NAT activity to light at night in Syrian hamsters.



Influence of time of day and of exposure to light at night on pineal N-acetyltransferase activity in the Syrian hamster. Units of N-acetyltransferase activity are defined as pmol N-acetyltryptamine formed in 1 h/pineal gland. •, hamsters killed from 16.00 h to 06.00 h in the normal lighting regimen, with lights off from 18.00 h to 06.00 h. O, hamsters exposed to light at 03.30 h and killed 30 min later with light on. Points are means \pm SEM of 4-5 determinations from 8-10 animals. The solid line indicates the duration of the dark period. Differences between groups were analyzed for significance using Student's t-test. Night N-acetyltransferase activity was significantly higher than the day activity measured at 16.00 h at 02.00 h (p < 0.001), 03.30 h (p < 0.001), 04.00 h (p < 0.02) and 05.00 h (p < 0.02). The activity in hamsters exposed to light at 03.30 h was significantly lower than the activity at 03.30 h (p < 0.001) and 04.00 h (p < 0.001) in darkness and did not differ significantly from the day value at 16.00 h. Male Syrian hamsters were housed from the age of 35 days in a room lit from 06.00 h to 18.00 h by 40 W Tesla fluorescent tubes for 3 weeks prior to the experiment. During exposure to light at night, the intensity of illumination at the level of cages was around 100 lx. Hamsters killed in darkness were exposed to a dim red light for less than 1 min prior to decapitation. Pineals were removed immediately and stored in petri dishes on solid CO₂. Within 24 h, 2 pineals were homogenized in 100 µl of 0.1 M sodium phosphate buffer, pH 6.8, containing 0.25 mM/1-¹⁴C/-acetyl CoA (sp. act. 1 Ci/mole) and 10 mM tryptamine, and NAT activity was determined by a modified method of Deguchi and Axelrod Blanks with boiled pineal homogenates were carried through the procedure. Acetyl-/1-¹⁴C/-coenzyme A (59 mCi/mmole) was purchased from Radiochemical Centre, Amersham, England.

NAT activity began to increase around 23.00 h and was maximal between 02.00 h and 05.00 h (fig.). The ratio between the highest night activity and the day activity at 16.00 h was 3.5. When hamsters were exposed to sudden light at 03.30 h, NAT activity declined within 30 min to almost ½ of its former value. The discrepancy between our results and those of Tamarkin et al. 13 may be due to a difference in NAT assay. Tamarkin et al. 14 homogenized pineals, in contrast to our method, only in a phosphate buffer, without acetyl CoA which stabilizes NAT 16. However, Panke et al. 12 used the same procedure and in spite of this found the NAT rhythm. The low NAT amplitude in Syrian hamsters may be due to lower activity as compared with rats or to the lack of optimization of assay conditions. NAT was assayed at the pH optimal for the rat enzyme 15; the pH optimal for the Syrian hamster enzyme is not known. Our demonstration in Syrian hamsters of the daily rhythm in pineal NAT activity and of the rapid NAT

decline in response to light at night together with the previous demonstration of the daily rhythm in melatonin content ^{12,13} and of the fast drop of pineal melatonin after light exposure ¹³ indicates that changes in NAT activity are involved in the regulation of the melatonin rhythm in Syrian hamsters as well.

- The authors are grateful to Mrs Marie Svobodová for her skillful technical assistance.
- 2 H. Weissbach, B.G. Redfield and J. Axelrod, Biochim. biophys. Acta 43, 352 (1960).
- 3 D.C. Klein and J.L. Weller, Science 169, 1093 (1970).
- 4 M. Wilkinson, J. Arendt, J. Bradtke and D. de Ziegler, J. Endocr. 72, 243 (1977).
- 5 D.C. Klein and J.L. Weller, Science 177, 532 (1972).
- 6 T. Deguchi and J. Axelrod, Proc. natl Acad. Sci. USA 69, 2547 (1972)
- H. Illnerová, M. Bäckström, J. Sääf, L. Wetterberg and B. Vangbo, Neurosci. Lett. 9, 189 (1978).
- 8 H. Illnerová, J. Vaněček, J. Křeček, L. Wetterberg and J. Sääf, J. Neurochem. 32, 673 (1979).
- 9 K. Hoffmann, H. Illnerová and J. Vaněček, Naturwissenschaften 67, 408 (1980).
- 10 K. Hoffmann, H. Illnerová and J. Vaněček, Biol. Reprod. 24, 551 (1981).
- 11 L. Tamarkin, S.M. Reppert, D.J. Orloff, D.C. Klein, S.M. Jellan and B.D. Goldman, Endocrinology 107, 1061 (1980).
- E.S. Panke, R.J. Reiter, M.D. Rollag and T.W. Panke, Endocr. Res. Commun. 5, 311 (1978).
- 13 L. Tamarkin, S.M. Reppert and D.C. Klein, Endocrinology 104, 385 (1979).
- 14 A. Parfitt, J.L. Weller, D.C. Klein, K.K. Sakai and B.H. Marks, Molec. Pharmac. 11, 241 (1975).
- 15 T. Deguchi and J. Axelrod, Analyt. Biochem. 50, 174 (1972).
- 16 S. Binkley, D.C. Klein and J.L. Weller, J. Neurochem. 26, 51 (1976).

Normal prolactin content of rat pituitary may be maintained by Nebenkern formations

C.W. Reifel and S.H. Shin

Departments of Anatomy and Physiology, Queen's University, Kingston (Ontario, Canada), 1 June 1981

Summary. We have observed Nebenkern formations in mammotrophs of normal male rats. The ultrastructural appearance of these formations suggests that they may be part of a mechanism which by the process of autophagy disposes of older prolactin granules.

The effect of either lactation^{1,2} or estrogen treatment³⁻⁵ on pituitary mammotrophs is reflected ultrastructurally by an extensive proliferation of the protein synthetic apparatus. The most striking feature is the presence of focal proliferations of rough endoplasmic reticulum (RER) in the formation of concentric whorls; the centers of such structures have been reported to contain dense granules surrounded by agranular membranes⁴, secretory granules and free ribosomes⁵, vesicles, dilated buds of RER, anastomosing tubules of smooth endoplasmic reticulum and lysosomes or multivesicular bodies⁶. Haguenau and Bernhard⁷ saw similar formations in mammotrophs from estrogen-induced pituitary tumors and named them 'Nebenkern'. Most authors refer to Nebenkern as structures associated with active protein synthesis¹. Only Pantic and Genbacev⁴ have suggested the possibility that Nebenkern represent a stage in the formation of bodies belonging to the lysosomal system. Pantic and Genbacev⁴ reported that following estrogen treatment, Nebenkern are more developed in male than in female rats, while in untreated male rats they are not present and the RER is only slightly developed.

Materials and methods. 5 young male rats were housed in group cages in a temperature and humidity controlled sound proofed room with controlled lighting regulated to 14 h light and 10 h dark. Animals were kept in this controlled environment for 3 weeks prior to sacrifice. Pituitary glands were fixed by immersion in glutaraldehyde/osmium tetroxide and embedded in Epon 812. Sections were cut on glass knives and stained with uranyl acetate followed by lead citrate.

Results. Mammotrophs were present in small numbers and were somewhat irregular in shape, often with long cytoplasmic processes (fig. 1). Nuclei were centrally located. The mammotroph shown displays a moderately developed protein synthetic apparatus in that there are several rows of elongated RER and a distinct Golgi apparatus. The small number of secretory granules and the presence of exocytotic figures (fig. 2) suggest that the cell is in a secretory phase. Concentrically arranged RER (Nebenkern) can be seen enclosing a dense granule and vesicle, free ribosomes and what appears to be a granule in a partial state of digestion (fig. 2). In other mammotrophs a lipid droplet was some-