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DAILY RHYTHMIC VARIATION AND LIVER DRUG METABOLISM IN RATS

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Abstract—Four substrates (hexobarbital, imipramine, *p*-nitroanisol and aminopyrine) are metabolized by rat liver (9000 *g* fraction) with a rhythm showing a minimum or a maximum between 10.00 a.m. and 2.00 p.m. The minimum was reached during light and the maximum during darkness when the illumination schedule was respectively from 6.30 a.m. to 6.30 p.m. or from 6.30 p.m. to 6.30 a.m.

The change in drug metabolism corresponds also to a change in the level of plasma corticosterone.

The possibility that neuroendocrine factors may be responsible for the daily rhythm of drug metabolism is discussed.

DAILY rhythmic variations for the *in vitro* metabolism of drugs by rat liver were firstly reported by Radzialowski and Bousquet.[1,2]

These biochemical data support, and at least partially explain, the well known observations that pharmacological effects[3–5] as well as blood levels of drugs such as hexobarbital,[6] methyrapone[7] and cortisol[8] are submitted to diurnal variability. The involvement of hormonal factors in the regulation of the daily rhythm in drug metabolism has been suggested, since variations in metabolic rate are in close relation to the variations of endogenous plasma corticosterone levels.[1,8,7] This report describes the alterations in the diurnal rhythmicity of drug metabolism activity and corticosterone plasma concentrations when the schedule of environmental lighting was artificially reversed. The possibility that various neuroendocrine systems may be implicated in the control of the daily fluctuations of drug metabolism is discussed.

MATERIALS AND METHODS

Female Long–Evans rats (obtained from the Laboratories Servier, Orléans, France) weighing 200 ± 20 g were used.

They were housed at 22° with a relative humidity of 60 per cent with food and water *ad lib.*

Rats were exposed to a daily schedule of 12 hr light and 12 hr darkness; one group of animals was at light between 6.30 a.m. and 6.30 p.m. while the second group was kept at darkness during the same time.

The rats were acclimatized to these cycles during 4 weeks before the experiments. Animals were sacrificed at 4-hr intervals. Plasma was collected and livers removed immediately, frozen on dry ice and stored at −20°. Livers were then homogenized and centrifuged at 9000 *g* for 30 min at 4°.

Determinations of the enzymatic activity were performed on the supernatant fraction incubated with cofactors and substrates according to Kato and Takanaka.[9]

The following substrates were used: hexobarbital (4 μmoles), imipramine (1 μmole), *p*-nitroanisol (1·5 μmoles) and aminopyrine (5 μmoles). Hexobarbital and imipramine disappearance were determined according to Cooper and Brodie[10] and Dingell *et al.*[11] respectively; *p*-nitrophenol (formed from *p*-nitroanisol) and 4-amino-antipyrine (formed from aminopyrine) according to Gilbert and Goldberg;[12] plasma corticosterone according to Guillemin *et al.*[13] with minor modifications.

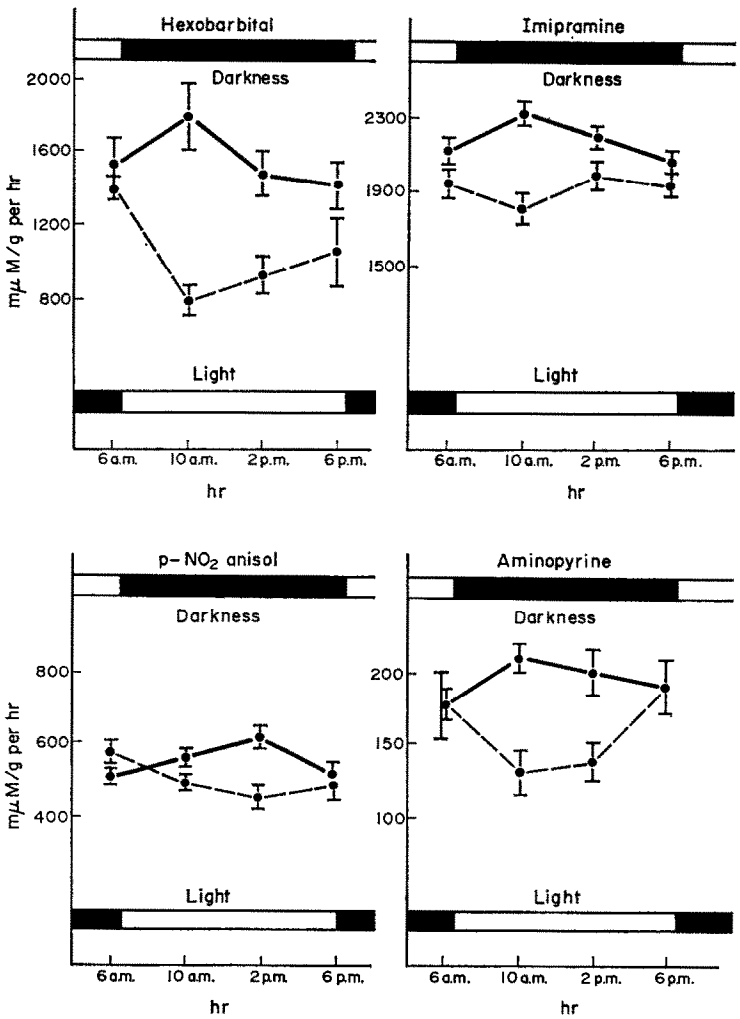

FIG. 1. Drug metabolizing enzymatic activity of the rat liver at different times of the day in different illumination conditions.

The enzymatic activity is expressed as mμmoles of product disappeared (hexobarbital and imipramine) or of the corresponding metabolites formed (pNO_2phenol from pNO_2anisol and $4HN_2$antipyrine from aminopyrine) by 9000 *g* supernatant fraction/g of liver/hr.

Vertical bars represent the Standard Error.

For each substrate the lower curve (dotted line) indicates the results obtained with rats maintained at the following illumination schedule: light between 6.30 a.m. and 6.30 p.m. and darkness between 6.30 p.m. and 6.30 a.m. The upper curve (continuous line) shows the results obtained when rats were exposed to darkness between 6.30 a.m. and 6.30 p.m. and to light between 6.30 p.m. and 6.30 a.m.

RESULTS

In conditions of normal illumination (from 6.30 a.m. to 6.30 p.m.) preparations of liver microsomal enzymes show a pattern with a minimum of activity between 10.00 a.m. and 2.00 p.m. This pattern is similar for the different enzymatic reactions studied such as *N*-demethylation (aminopyrine and imipramine), alkylhydroxylation (hexobarbital) and *O*-demethylation (*p*-NO_2 anisol).

A mirror image of this rhythm in drug metabolism is obtained when the alternating periods of light and darkness were reversed. (See Fig. 1.)

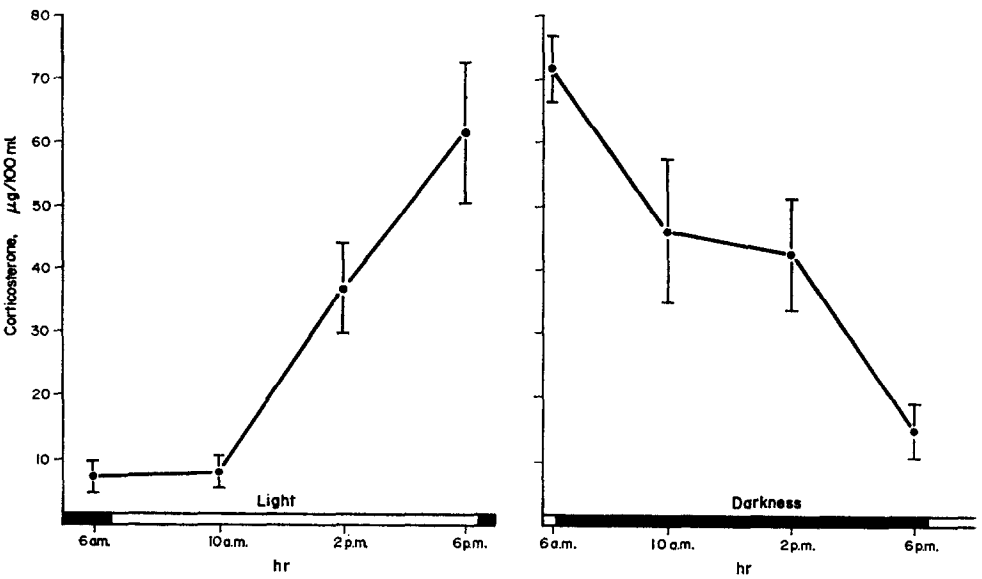

FIG. 2. Plasma corticosterone levels at different times of the day in different illumination conditions. On the left: light between 6.30 a.m. and 6.30 p.m. and darkness between 6.30 p.m. and 6.30 a.m.; on the right: darkness between 6.30 a.m. and 6.30 p.m. and light between 6.30 p.m. and 6.30 a.m.

Similar results were obtained when plasma corticosterone levels were measured. Figure 2 shows in fact that plasma corticosterone levels increase between 6.00 a.m. and 6.00 p.m. when the animals are kept in lightness while they decrease during the same time of the day, when the cycle of the illumination was reversed

DISCUSSION

The data reported in this investigation, confirm that the rate of drug metabolism by liver microsomal enzymes follows a daily rhythm. According to Radzialowski and Bousquet[2] the minimum of the activity for the metabolism of aminopyrine and pNO_2 anisol was observed about at 2 p.m. while in our experimental conditions the minimum value for hexobarbital was attained 4 hr before. Except some minor discrepancies about the hours, all the authors found a minimum of activity during the day and a peak during the night.[2,6] Nair and Casper[6] observed that by exposing rats to a constant light, the rhythmicity of the hepatic metabolism is abolished and the enzymatic activity is stabilized at a low rate. On the contrary after exposure at continuous darkness the animals showed a higher level of metabolic activity. In our experiments the

artificial reversal of day–night illumination sequence, completely reverses the rhythm for the metabolism of all the substrates studied, producing a peak of enzyme activity at the same hour exhibiting the minimum in the normal illumination conditions.

The daily schedule of 12 hr light and 12 hr darkness used in this study insures against stressing situations such as continuous darkness or lighting which could modify drug metabolism independently from the suggested correlation between enzyme activity and diurnal light alternance.

In addition, our studies show that also the daily variations in the endogenous levels of plasma corticosterone that are extensively documented in rats[14–16] may be reversed when the cycles of light–darkness are also inverted. According to other authors[8,17,18] the peak in plasma corticosterone occurs at 6.00 p.m. in normal illumination sequence and when the illumination cycle is artificially reversed, this peak appears at 6.00 a.m.[19] Radzialowski and Bousquet[2] showed a disappearance of the daily rhythm in drug metabolism when the rats were previously adrenalectomized or maintained at constant corticosterone levels suggesting that the adrenal gland through corticosterone secretion may regulate the daily rhythm in oxidative drug metabolism. However, the possibility that the lighting-dependent microsomal enzymatic activity is under the control of a more central hormonal factor must not be ruled out.

Nair *et al.*[20] reported the importance of the hypothalamo-hypophyseo-adrenal axis for drug metabolism showing that the hexobarbital oxydase activity is reduced in head irradiated or hypophysectomized or hypothalamic lesioned rats. It is also interesting to recall that corticotropine, as well as corticosterone, exhibits a daily rhythm[21] that may be abolished by continuous lighting.[18] Guillemin[22] suggested that the corticotropine rhythmicity could be regulated by the secretion of the hypothalamic releasing factor for corticotropine. In fact continuous light modifies the release of several hypothalamic hormone releasing factors (HRF).[23,24] More recently Reiter[25] suggested an interaction of the hypothalamo-hypophyseo-gonadal axis in light-gonadal relationship with pineal melatonin.

It is well known that melatonin which is synthetized in large extent during darkness[26] exerts a regulatory control on estrogenic activity.[27] Since the role of sexual hormones in regulating drug metabolism in rat has been extensively reported[28–30] it may be suggested that melatonin could be another neurohormone that may play a role in the lighting dependent microsomal activity. As far as man is concerned it is interesting to recall here that endogenous cortisol half-life in plasma is shorter in the afternoon than in the morning.[31] Further studies on the relationship between neurohormonal activity and drug metabolism will be necessary. On the basis of our data at present no conclusions may be drawn but it may be pointed out the importance of lighting schedule as one of the environmental factors responsible for the variations in drug metabolism in rats and possibly in other animal species.

REFERENCES

1. F. M. RADZIALOWSKI and W. F. BOUSQUET, *Life Sci.* **6**, 2545 (1967).
2. F. M. RADZIALOWSKI and W. F. BOUSQUET, *J. Pharmac. exp. Ther.* **163**, 229 (1968).
3. F. E. HALBERG and A. STEPHENS, *Fedn Proc. Fedn Am. Socs. exp. Biol.* **18**, 63 (1959).
4. W. M. DAVIS, *Experientia* **18**, 235 (1962).
5. E. F. LUTSCH and R. W. MORRIS, *Science* **156**, 100 (1967).
6. V. NAIR and R. CASPER, *Life Sci.* **8**, 1291 (1969).
7. S. SZEBERENYI, K. SZ. SZALAY and S. GARATTINI, *Biochem. Pharmac.* **18**, 2767 (1969).
8. V. MARC and P. L. MORSELLI, *J. Pharm. Pharmac.* **21**, 864 (1969).

9. R. KATO and A. TAKANAKA, *Jap. J. Pharmac.* **17,** 208 (1967).
10. J. R. COOPER and B. B. BRODIE, *J. Pharmac. exp. Ther.* **114,** 409 (1955).
11. J. V. DINGELL, F. SULSER and J. R. GILLETTE, *J. Pharmac. exp. Ther.* **143,** 14 (1964).
12. D. GILBERT and L. GOLDBERG, *Food & Cosmet. Toxicol.* **3,** 417 (1965).
13. R. GUILLEMIN, G. W. CLAYTON and H. S. LIPSCOMB, *J. Lab. clin. Med.* **53,** 830 (1959).
14. F. HALBERG, E. HALBERG, C. P. BARNUM and J. J. BITTNER, *Publ. Am. Ass. Advance Sci.* n.55, p. 803 (1959).
15. V. CRITCHLOW, R. A. LIEBERT, M. BAR-SELA, W. MOUNTCASTLE and H. S. LIPSCOMB, *Am. J. Physiol.* **205,** 807 (1963).
16. C. ALLEN and J. W. KENDALL, *Endocrinology* **80,** 926 (1967).
17. R. GUILLEMIN, W. E. DEAR and R. A. LIEBELT, *Proc. Soc. exp. Biol. Med.* **101,** 394 (1959).
18. P. CHEIFETZ, N. GAFFUD and J. F. DINGMAN, *Endocrinology* **82,** 1117 (1968).
19. B. N. DIXIT and J. P. BUCKLEY, *Life Sci.* **6,** 755 (1967).
20. V. NAIR, T. BROWN, D. BAU and S. SIEGEL, *Eur. J. Pharmac.* **9,** 31 (1970).
21. J. H. GALICICH, F. HALBERG, L. A. FRENCH and F. UNGAR, *Endocrinology* **76,** 895 (1965).
22. R. GUILLEMIN, *Recent Prog. Horm. Res.* **20,** 89 (1964).
23. A. NEGRO-VILAR, E. DICKERMAN and J. MEITES, *Endocrinology* **82,** 939 (1968).
24. B. E. PIACSEK and J. MEITES, *Fedn Proc. Fedn Am. Socs. exp. Biol.* **25,** 191 (1966).
25. R. J. REITER, *Gen. Comp. Endocr.* **12,** 460 (1969).
26. R. J. WURTMAN, J. AXELROD and L. PHILIPS, *Science* **142,** 1071 (1963).
27. R. J. WURTMAN, J. AXELROD and E. W. CHU, *Science* **141,** 277 (1963).
28. G. P. QUINN, J. AXELROD and B. B. BRODIE, *Biochem. Pharmac.* **1,** 152 (1958).
29. R. KATO and J. R. GILLETTE, *J. Pharmac. exp. Ther.* **150,** 285 (1965).
30. D. S. DAVIES, P. L. GIGON and J. R. GILLETTE, *Biochem. Pharmac.* **17,** 1865 (1968).
31. P. L. MORSELLI, V. MARC, S. GARATTINI and M. ZACCALA, *Biochem. Pharmac.* **19,** 1643 (1970).