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CIRCADIAN RHYTHM OF THE INHIBITORY EFFECT OF ACTINOMYCIN D ON CYTOCHROME P-448 INDUCTION BY 3-METHYLCHOLANTHRENE

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Induction of cytochrome P-448 by polycyclic hydrocarbons has been shown by experimental use of actinomycin D (AD) to depend on de novo RNA synthesis (1-3). During the course of administration of 3-methylcholanthrene (MC) to rats, RNA polymerase activity (4) as well as the incorporation of radiolabelled precursors into total intranuclear RNA (4), poly-A-containing RNA (5) and 18 S and 28 S cytoplasmic RNA (6) is increased in the liver cell. A cytoplasmic receptor for cytochrome P-448 induction has recently been described to be translocated into the nucleus and is assumed to initiate gene expression by some way not yet understood (7). Processes involved in the control of induction of cytochrome P-448 are also found beyond the level of transcription. One of the first events during MC treatment of rats is an increase of activity of the initiation factors of protein synthesis, IF-M_2A and IF-M_2B; this increase preceeds measurable increase of transcription (8). The present report provides evidence that the extent to which the induction of cytochrome P-448 is controlled on the level of transcription depends on a circadian rhythm of the liver cell.

The experiments were performed with male Sprague-Dawley rats weighing 150-180 g which received a single i.p. dose of 20 mg MC/kg dissolved in peanut oil 24 hr before death. MC treatment was started either at 10.00 hr or at 20.00 hr. AD was administered i.p. in three doses of 0.75 mg/kg dissolved in 10 % ethanol at 1 hr before, 4 hr after and 12 hr after the application of MC. Several control groups were run receiving (a) PB as an inducer at two doses of 80 mg/kg, dissolved in 0.9 % NaCl 24 and 12 hr before death, treatment starting either at 10.00 hr or at 20.00 hr, with and without the AD protocol described for MC induction, (b) cycloheximide as an inhibitor instead of AD, administered at 4 i.p. doses of 1 mg/kg dissolved in 0.9 % NaCl, at 1 hr before and 6, 12, and 18 hr after start of inducer treatment with either MC or PB, (c) treatment with AD or cycloheximide at the protocol described above but without inducer, (d) treatment with the solvents of both the inducers and inhibitors at the appropriate times. Preparation of microsomes, SDS-PAGE and spectral and enzymic measurements were performed as described previously (9-11).

The effect of AD on MC induction is shown in Tab. 1 and 2. MC administration leads to similar increases in enzyme concentration and monooxygenase activity when started at 10 hr or 20 hr. The following parameters indicate the induction: (a) increase in total concentration of cytochromes P-450, (b) a 2 nm blue shift of the CO spectrum of the reduced microsomes, (c) an affinity loss of the reduced cytochromes for the ligand metyrapone, (d) a large increase in biphenyl-2-hydroxylation, (e) a slight increase in biphenyl-4-hydroxylation, (f) a change in the gelelectrophoretic peptide pattern in the hemoprotein region between 48 000 and 60 000 dalton. Increase of band 1 (51 000 dalton) is associated with PB induction, while bands 2 (54 000 dalton) and 3 (57 000 dalton) can be increased by treatment with polycyclic hydrocarbons (12). In this study, the proportion of band 3 is already high in control liver,

Table 1 Circadian rhythm of the influence of actinomycin D (AD) and cycloheximide (CH) on cytochrome P-448 induction by 3-methylcholanthrene (MC): Spectral and enzymic properties of liver microsomes.

Pretreatment	Start of inducer treatment	n	Cytochrome P-450 content (nmol/mg protein)	λ_{max} (nm)	K_s for metyrapone (μM)	Biphenyl-2-hydroxylation (nMol/mg protein x min)	Biphenyl-4-hydroxylation (nMol/mg protein x min)
Oil	20 hr	3	1.05 ± 0.02	449.8 ± 0.17	89 ± 17	0.11 ± 0.04	2.37 ± 0.29
MC	20 hr	3	1.45 ± 0.02**	448 ± 0	277 ± 49*	1.30 ± 0.04**	4.12 ± 0.26*
AD + MC	20 hr	3	1.43 ± 0.08**	449 ± 0	226 ± 21**	0.68 ± 0.05**	4.15 ± 0.07**
CH + MC	20 hr	2	1.08 / 0.87	450 / 450	89 / 55	0.12 / 0.20	2.70 / 1.77
MC	10 hr	3	1.45 ± 0.01**	448 ± 0	297 ± 39**	1.20 ± 0.11**	3.82 ± 0.33
AD + MC	10 hr	3	1.05 ± 0.05	449.5 ± 0	89 ± 23	0.23 ± 0.03	2.79 ± 0.16
CH + MC	10 hr	1	1.02	450	65	0.11	2.39

Table 2 Circadian rhythm of the influence of actinomycin D (AD) and cycloheximide (CH) on cytochrome P-448 induction by 3-methylcholanthrene: Densitometry of molecular weight region between 48 000 and 60 000 in SDS polyacrylamide gels of liver microsomes.

Pretreatment	Start of inducer treatment	n	Band 1 (%) (mol.wt. 51 000)	Band 2 (%) (mol.wt. 54 000)	Band 3 (%) (mol.wt. 57 000)
Oil	20 hr	3	33 ± 3	10 ± 1	20 ± 1
MC	20 hr	3	21 ± 1*	22 ± 1**	22 ± 1
AD + MC	20 hr	3	23 ± 1*	21 ± 1**	19 ± 1
CH + MC	20 hr	2	30 / 19	12 / 12	21 / 21
MC	10 hr	3	23 ± 2*	23 ± 1**	24 ± 2
AD + MC	10 hr	3	41 ± 4	12 ± 1	19 ± 1
CH + MC	10 hr	1	32	11	22

Values of Tab. 1 and 2 are means ± S.E. * $P < 0.05$, ** $P < 0.01$ against oil group. In all experiments food and water were available ad libitum.

and no increase is obvious by MC treatment. However, band 2 is distinctly increased by a factor of 2.

While the timing of induction start has no effect on the extent of cytochrome P-448 formed, it did profoundly influence the effect of AD on the induction process. When treatment started at 10.oo hr, AD completely prevented any inducing action of MC. However, when treatment started at 20.oo hr, almost no inhibitory effect of AD was observed. In this case, the only data different from those obtained by MC treatment in the absence of the inhibitor were (a) less marked blue shift and (b) less marked induction of biphenyl-2-hydroxylation.

When the protein synthesis inhibitor cycloheximide was used instead of AD, this circadian rhythm of inhibition was not obtained. Complete prevention of MC induction was found independently from the time of starting the treatment. Also, when PB was used as the inducer instead of MC, a circadian rhythm of the inhibitory effect of AD was not observed; instead, complete inhibition was obtained by both timing protocols (data not shown). No influence of

timing was apparent in control animals treated with oil, with AD, and with cycloheximide.

These results show that the inhibitor of RNA synthesis, AD, effectively blocks MC induction when treatment starts in the morning but is unable to effectively inhibit MC induction when treatment starts in the evening. Two reasons may hold for the failure of AD to inhibit the induction of cytochrome P-448 when first administered in the evening: 1) AD may in general be much less effective in the evening than in the morning. 2) Cytochrome P-448 induction may not depend on de novo RNA synthesis when started in the evening.

As far as we know, there is no evidence from the literature as to a circadian rhythm of AD action on transcription. However, there are data which allow the possibility that such a rhythm may exist. For instance, the binding capacity of DNA for AD is higher by a factor of 2 at 15 hr than at 9 hr (13), and possibly not all of the sequences blocked by AD intercalation at 9 hr can also be blocked by the same dose of AD in the afternoon. Also, drug metabolism itself exhibits a circadian rhythm with a nocturnal maximum (14), and the first AD dose may have been critically impaired by biotransformation during the protocol starting treatment in the evening. The different feeding state of the two groups at start of treatment may have influenced AD action; however, no such effect was observed in the parallel PB induction experiments.

However, if there is no general impairment of the effectivity of AD in the evening, the present results may have important implications for the mechanism of cytochrome P-448 induction. They confirm that this process is regulated at two levels, during transcription and by posttranscriptional steps. From our data it seems possible that not always inducer-directed de novo RNA synthesis is required for the formation of cytochrome P-448. Instead, the RNA species necessary for its synthesis may be formed constitutively at a rate which shows a circadian rhythm. Formation may be low in the morning, and it may be a main function of the inducer at that time of the day to enhance transcription. However, in the night when RNA synthesis in the rodent liver is increased, possibly under the influence of steroids (15), enough of those RNA molecules required for cytochrome P-448 synthesis may be provided, and the main function of the inducer in this situation should then be the release of some posttranscriptional control, f.i. by activation of initiation factors of protein synthesis (8) or other control mechanisms operative on the level of RNA maturation and discrimination or on the level of translation (for review see 16). Experiments are in progress in our laboratory to clarify if the failure of AD in the evening is a more general phenomenon or if it is restricted to cytochrome P-448 synthesis and does, therefore, implicate the proposed concept of induction.

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