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THE ROLE OF TESTOSTERONE IN THE REGULATION OF OXIDATIVE DRUG METABOLISM IN NORMAL AND IRRADIATED ANIMALS

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Abstract—The rate of oxidative demethylation of aminopyrine in the liver endoplasmic reticulum of male rats is inhibited by whole body irradiation (850 rad). It reaches a minimum on days 4–5 following irradiation when the V_{max} is decreased and the K_m markedly increased. Irradiation does not affect the lower rate of oxidative demethylation in female rats. Irradiation of the testis only with 1000 or 1500 rad also caused a decrease of V_{max} and a large increase of K_m for oxidative demethylation in the liver. Injection of testosterone following irradiation restores the K_m and V_{max} in male rats nearly to control values. Phenobarbitone injection following irradiation restores the K_m for the oxidative demethylation to normal values and also causes a large increase in V_{max} in both control and irradiated animals. Methyl cholanthrene injection following whole body irradiation does not affect the rate of oxidative demethylation of aminopyrine but increases the rate of hydroxylation of biphenyl to very high values equal to those observed after injection of unirradiated animals. It is considered that whole body irradiation does not affect the liver endoplasmic reticulum directly but inhibition of oxidative demethylation is caused by an inhibition of the synthesis, or release of testosterone from the testes.

OXIDATIVE demethylation of drugs such as pethidine or aminopyrine in the liver is depressed after whole body irradiation of male rats, minimum activity being observed 3–5 days following irradiation.[1,2]

The slower rate of oxidative drug metabolism in female rats is, however, not affected by irradiation.[2] Nair and Bau[3] showed that irradiation to the head only caused inhibition of liver drug metabolism. It is therefore possible that the effect on the liver observed after whole body irradiation is indirect, and caused by a disturbance of a hormonal regulatory system.

The importance of hormones, in particular testosterone, was demonstrated by Kato and Onoda[4] who showed that the rate of drug oxidation in male rat liver microsomes was strongly inhibited following castration.

In this paper we describe kinetic studies of oxidative drug metabolism and induction of the endoplasmic reticulum by drugs and carcinogens in normal and irradiated animals which demonstrate the important role which testosterone plays in controlling this metabolism in normal male animals.

MATERIALS AND METHODS

Animals. Wistar rats (Scientific Animal Supplies), 8–10 weeks old, weighing 150 g on average, were used in all experiments.

Diet. Animals were fed on stock diet. MRC 41B.

Materials. Sources of most materials have been previously described.[5,6] Phenobarbitone was obtained from John Bell and Croyden Ltd., London, and 20-methyl cholanthrene from Koch-Light Ltd., Colnbrook, Bucks. Testosterone propionate dissolved in arachis oil was obtained from Organon Laboratories, Surrey.

Methods. The preparation of the microsomal fraction has been previously described[5] and this fraction was used for the study of the oxidative demethylation of aminopyrine and hydroxylation of biphenyl.

The rate of oxidative demethylation of aminopyrine was determined as previously described.[5,6] In most experiments designed to measure K_m and V_{max} the nicotinamide concentration was reduced to 5 mM to avoid competition with the substrate.[7]

Determination of biphenyl hydroxylase activity was carried out by the method of Creaven *et al.*[8]

Irradiations. These were carried out using a Cobalt 60 source under conditions previously described.[2] Whole body doses of 850 rad were normally given but doses of 1000 or 1500 rad were given in most experiments to the lower trunk.

RESULTS

Effect of whole body irradiation on V_{max} *and* K_m *for oxidative demethylation in male and female rats.* At daily intervals after a whole body dose of 850 rad, preparations of liver microsomes were made from groups of male rats and the rate of oxidative demethylation of aminopyrine measured. Five substrate concentrations between 10 and 0·25 mM were used to enable K_m and V_{max} to be determined by means of a Lineweaver–Burke plot.

The rate of oxidative demethylation fell after irradiation, reaching a minimum on days 4–5. Although this decrease was caused partially by a reduction in the V_{max}, a large increase in the K_m was a major contributory factor (Table 1).

Groups of female rats were also irradiated and rates of oxidative demethylation measured as for males. The rate in females was much less than in males and was little affected by irradiation (Table 1). It is significant that irradiation caused the V_{max} in males to fall to a value close to that of females (Table 1).

Effect of testosterone following irradiation. The sharp decline of oxidative demethylation following irradiation of males which was not observed in females indicated that a depression of testosterone secretion by the testis might be responsible.

To test this possibility 15 mg testosterone propionate was injected on the days 1–3 following irradiation. The rate of oxidative demethylation was then measured on days 3–5 following irradiation. Lineweaver–Burke plots of $1/v$ against $1/s$ demonstrated that this injection of testosterone was able to restore the gradient nearly to the control, and following the injection the V_{max} and K_m values were restored nearly to control values (Fig. 1). Unirradiated rats were also injected with testosterone and the rate of oxidative demethylation measured on subsequent days as described for irradiated animals. Very small increases in V_{max} and decreases in K_m were observed but these changes were not outside the range observed for the control animals.

Effect of irradiation of the lower trunk. Inhibition of testosterone synthesis following whole body irradiation could be a result of direct effect of irradiation on the testes and to test this hypothesis male rats were subjected to irradiation of the lower trunk region. The rats were first anaesthetized with nembutal (90 mg/kg), placed in perspex

TABLE 1. EFFECT OF WHOLE BODY IRRADIATION (850 rad) ON V_{max} AND K_m FOR OXIDATIVE DEMETHYLATION OF AMINOPYRINE IN MALE AND FEMALE RATS

Days after irradiation	Males		Females
	V_{max}	K_m	V_{max}
0 (Control)	5·41 ± 0·08	0·52 ± 0·034	1·19 ± 0·12
2	3·82 ± 0·27	0·50 ± 0·05	1·05 ± 0·10
3	2·92 ± 0·05	1·12 ± 0·10	..
4	1·49 ± 0·14	1·54 ± 0·12	1·25 ± 0·10
5	1·97 ± 0·12	1·48 ± 0·18	1·25 ± 0·15
6	3·44 ± 0·13	1·42 ± 0·10	1·23 ± 0·14
8	4·39 ± 0·20	0·88 ± 0·04	1·20 ± 0·10

V_{max} = nmoles formaldehyde/mg protein/min.
K_m = aminopyrine concentration (mM).
Standard errors are shown.

cages and the whole of the body from a line just anterior to the testis shielded with a lead block, 5 cm thick, which decreases the dose by $(0{\cdot}5)^5$ to about 31 rad for each 1000 rad given.

The testis and the lower limbs were given a dose of 1000 or 1500 rad but the remainder of the body, shielded by the lead, only received approx. 31/1000 rad given to the lower trunk. Previous experiments,[2,3] indicated that the dose given to the shielded area would have a negligible effect on the drug metabolism studies.

Rates of oxidative demethylation of aminopyrine were measured on days 3–5 following irradiation.

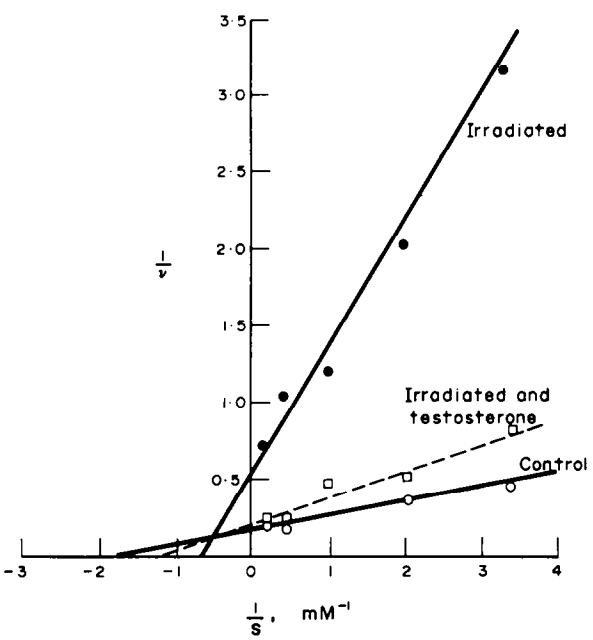

FIG. 1. Plot of $1/v$ against $1/s$ for the oxidative demethylation of aminopyrine by male rat liver microsomal preparations following whole body irradiation (850 rad) and testosterone injection. (○) Control; (●) day 4 following whole body dose of 850 rad; (□) day 4 following whole body dose of 850 rad. Testosterone (15 mg) injected on days 2 and 3 following irradiation. Values are means of four separate experiments.

TABLE 2. EFFECT OF IRRADIATING THE TESTIS ON THE OXIDATIVE DEMETHYLATION OF AMINOPYRINE BY LIVER MICROSOMAL PREPARATIONS

Days after irradiation	K_m		V_{max}
	850 rad	1500 rad	1500 rad
0 (Control)	0·52 ± 0·034		5·41 ± 0·076
3	0·65 ± 0·05	0·89 ± 0·05	5·10 ± 0·21
4	0·92 ± 0·04	1·25 ± 0·04	4·02 ± 0·42
5	0·64 ± 0·06	0·63 ± 0·05	4·56 ± 0·75
6	0·58 ± 0·07	0·57 ± 0·03	5·65 ± 0·44

Standard errors are shown.

The irradiation of the testis caused a marked increase in the K_m and a smaller decrease of V_{max} (Table 2).

Induction by phenobarbitone. The possibility that the rate of oxidative demethylation following irradiation could be restored by injection of drugs such as phenobarbitone was studied.

Groups of 12 male rats were given whole body doses of 850 rad and injected (i.p.) with a solution of phenobarbitone (15 mg) in sodium chloride (0·9 per cent) 1 hr after irradiation and at 24 hr intervals to give a total of three injections. Other groups were also injected but not irradiated and results were compared with control animals and those which had been irradiated but not injected.

At daily intervals determinations of the rate of oxidative demethylation of aminopyrine in liver microsomal preparations were carried out as described. Induction by phenobarbitone was not significantly different in normal and irradiated rats (Fig. 2).

The K_m and V_{max} for the reaction were not significantly different in the control and

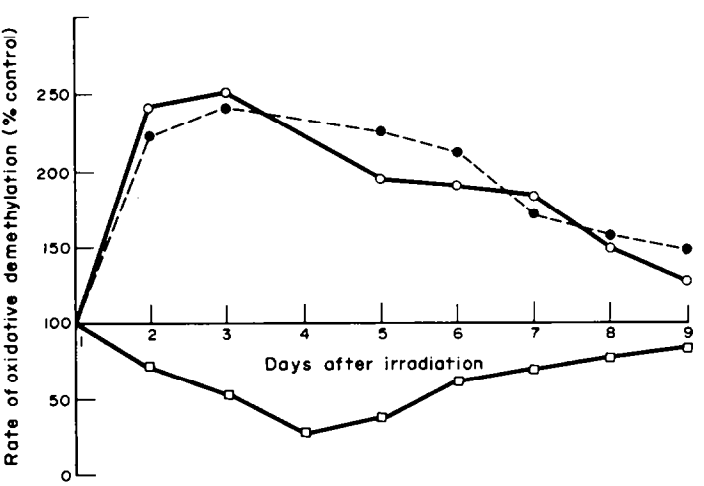

FIG. 2. Effect of injecting phenobarbitone on the rate of oxidative demethylation of aminopyrine in the liver endoplasmic reticulum of male rats after a whole body dose of 850 rad. Enzyme velocities are calculated as a percentage of the mean of 12 control experiments. (□) Irradiated; (●) irradiated and injected with phenobarbitone (15 mg) 1, 24 and 48 hr after irradiation; (○) injected with phenobarbitone at the same times as the irradiated group. Each point represents a mean of three experiments.

TABLE 3. EFFECT OF IRRADIATION (850 rad) AND PHENOBARBITONE INJECTION ON K_m AND V_{max} FOR THE OXIDATIVE DEMETHYLATION OF AMINOPYRINE BY LIVER ENDOPLASMIC RETICULUM OF MALE RATS

	K_m (mM)	V_{max} (nmoles formaldehyde /mg protein/min)
Control	0·52 ± 0·034	5·40 ± 0·076
Irradiated	1·60 ± 0·026	2·71 ± 0·16
Phenobarbitone injected	0·65 ± 0·030	13·50 ± 0·34
Phenobarbitone injected + irradiation	0·56 ± 0·034	13·05 ± 0·49

Values for irradiated animals are means of results obtained on days 3–5 following irradiation.
Phenobarbitone (15 mg) was injected 1, 24 and 48 hr after irradiation and values for phenobarbitone injected animals are means for days 3 and 5 following irradiation.
Standard errors are shown.

irradiated group following irradiation whereas in the non-irradiated group V_{max} was depressed and K_m increased by irradiation (Table 3).

Induction by methyl cholanthrene. Methyl cholanthrene, unlike phenobarbitone and other inducers, does not cause proliferation of the endoplasmic reticulum membrane, but acts as a more specific inducing agent.[9] The rate of *N*-demethylation of aminopyrine is not affected by methyl cholanthrene injection but the rate of the hydroxylation of the biphenyl ring is increased several times.[10]

Both hydroxylation of biphenyl and oxidative demethylation of aminopyrine are inhibited by irradiation of male rats[2] and we therefore investigated whether methyl cholanthrene could reactivate metabolism of either compound after irradiation.

Male rats were divided into two groups. One group was given 850 rad of whole body irradiation and the other group was used as a control. On the second day following irradiation half of the control group and half of the irradiated group were given 20 mg methyl cholanthrene in arachis oil (0·5 ml) intraperitoneally and this was continued until day 8.

At daily intervals the animals were killed, preparations of microsomes were made from their livers and the rates of oxidative demethylation of aminopyrine and hydroxylation of biphenyl measured.

Methyl cholanthrene injection had no effect on the inhibition of aminopyrine demethylation caused by irradiation (Table 4) but stimulation of hydroxylation of biphenyl both in the 2nd and 4th position occurred. This stimulation was especially marked for the 2-hydroxylation and stimulated rates in irradiated animals were sometimes greater than in controls.

DISCUSSION

These experiments, together with our previous observations, indicate that it is extremely unlikely that the whole body irradiation has any direct effect on the liver metabolising enzymes.

No inhibition of the system was observed in females (Table 1) and if suspensions of liver microsomes are irradiated *in vitro* large doses, greater than 2000 rad, are necessary to inhibit the enzyme system.[11]

It would, therefore, appear that the high activity of oxidative drug metabolizing enzymes in the male rat is dependent primarily on a supply of testosterone and that

TABLE 4. THE EFFECT OF METHYL CHOLANTHRENE ON THE RATE OF OXIDATIVE DEMETHYLATION OF AMINOPYRINE AND HYDROXYLATION OF BIPHENYL IN MALE RATS FOLLOWING 850 rad WHOLE BODY IRRADIATION

Days after irradiation	Rate of oxidative demethylation of aminopyrine (nmoles formaldehyde /mg protein/min)		Rate of 4-hydroxylation of biphenyl (nmoles/mg protein/min)		Rate of 2-hydroxylation of biphenyl (nmoles/mg protein/min)	
	Untreated	Injected with Me-cholanthrene	Untreated	Injected with Me-cholanthrene	Untreated	Injected with Me-cholanthrene
0 (Control)	5·33 ± 0·33	4·72 ± 0·28	7·87 ± 0·23	10·79 ± 0·59	0·73 ± 0·01	5·07 ± 0·23
2	3·84 ± 0·28	3·71	5·43 ± 0·31	12·90	0·64 ± 0·02	6·25
5	1·90 ± 0·12	1·90	2·50 ± 0·16	14·60	0·25 ± 0·05	6·38
8	4·16 ± 0·20	5·20	4·95 ± 0·30	12·38	0·39 ± 0·02	5·63

Standard errors are shown for control and irradiated animals. Other values for irradiated animals are means of three separate experiments.

the inhibition observed following irradiation is a result of blockage of supply of testosterone. Several observations support this view. Firstly, the decline of drug metabolism in male rats to the level of that in the females (Table 1) and secondly, the ability of testosterone to restore the activity of oxidative demethylation to normal in irradiated animals (Fig. 1). The effect of irradiation of the whole body on liver drug metabolism is thus very similar to that observed following castration of male rats.[4]

It is interesting that the dose of testosterone used had little effect on the K_m or V_{max} measured for the controls. Testosterone was therefore able to restore control rates in irradiated animals but not to cause the marked stimulation in V_{max} observed following phenobarbitone injection. It is possible that this is partially due to a rapid rate of metabolism of testosterone.

It is particularly significant that following irradiation a marked decrease of K_m for the system occurs. It is difficult to interpret this precise significance of changes in K_m for such a complex system because the rate of the reaction is dependent on the operation of a complex electron transport system. Furthermore, the K_m measured experimentally depends on the rate of formation of enzyme–substrate complex (k_1) the rate of breakdown of the complex to enzyme + substrate (k_{-1}) and the rate of formation of the product of the reaction (k_{cat}). Changes in any or all of these parameters could cause a change in the value of K_m. There is good evidence however that before oxidation, drugs must be bound to cytochrome P-450. A decrease of K_m following irradiation, and a restoration to normal after testosterone injection, suggest that testosterone may play a vital role in regulation of the affinity of cytochrome P-450 for its drug substrates.

The fact that irradiation of the testis only, caused a marked depression of oxidative drug metabolism (Table 2) implies that during whole body irradiation the testis may be directly damaged, so that the secretion of testosterone is suppressed. However, the secretion of testosterone is under the control of the pituitary gonadotrophins, and especially LH, and thus indirectly controlled by the hypothalamus. It is thus possible that following whole body irradiation, effects on liver drug metabolism could be caused by a depression of the liberation of LH and some experiments in which the head only was irradiated tend to support this view.[3]

Although oxidative demethylation of aminopyrine was strongly inhibited by irradiation, the K_m was increased and V_{max} depressed, the activity following phenobarbitone injection was increased to that of the controls (Fig. 1). These induction experiments also support the concept that irradiation does not damage the enzymes of the microsomal electron transport chain. This induction process involves synthesis of membrane phospholipids, membrane enzymes, cytochromes and other proteins, and the induction is inhibited by puromycin.[12] It is also inhibited by actinomycin[12] and thus may involve the transcription of new messenger RNA. The life span of the *m*-RNA in slowly dividing cells such as the liver may be long and enable protein synthesis to be induced without frequent transcription of *m*-RNA but recent experiments on induction in cultured mammalian cells support the concept of new messenger RNA synthesis during the induction process.[13]

Many investigations in radiobiology have been interpreted on the basis that DNA damage is a primary and essential consequence of irradiation of living cells.[14] However, if *m*-RNA must be newly transcribed during the induction process, then DNA cannot be damaged by the dose of irradiation used (850 rad). Alternatively, damage

to DNA must be rapidly repaired because permanent damage to DNA would cause inhibition of *m*-RNA formation or its malformation during the induction process.

The inhibition of the hydroxylation of biphenyl caused by irradiation also responds to injection of methyl cholanthrene (Table 4). Oxidative demethylation of aminopyrine was not, however, restored following irradiation by injection of methyl cholanthrene and the enzyme activity is not induced by methyl cholanthrene in normal rats.[10] These experiments, therefore, support the view that hydroxylation of molecules of the biphenyl type depends on an enzyme system which is different from that necessary for oxidative demethylation and which is induced by a more limited range of inducing drugs or hydrocarbons.

It may, therefore, be concluded that the high level of oxidative drug metabolism in male rats is a result of a supply of the natural substrate and inducer testosterone, and it is inhibition of the supply of testosterone, either directly, by inhibition of its synthesis or release from the testis, or indirectly, by an effect on the pituitary or hypothalamus that cause an inhibition of oxidative drug metabolism following whole body irradiation.

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