THE BINDING OF ETHYL CARBAMATE TO LIVER DNA IN GONADECTOMISED MICE

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SUMMARY

Castration, with or without subsequent hormone administration resulted in different levels of binding of a metabolite of ethyl carbamate to mouse liver DNA. In ovariectomised and ovariectomy plus testosterone-treated females, the binding persisted for longer than in intact and ovariectomy plus estradiol-treated females, although the maximum levels of binding were similar in all four groups. In males, orchidectomy dramatically lowered the level of binding compared with that in intact males. Twelve hours after dosing, the levels of binding were 276 and 9.8 nanomoles g. DNA^{-1} respectively in the two groups. Estradiol treatment did not restore the level of binding whereas testosterone treatment did.

INTRODUCTION

A metabolite of the carcinogen ethyl carbamate (urethan) probably an ethyl or ethoxy group, binds to liver DNA, RNA and protein (1). Because of the low levels of binding to and the

rapid loss of bound material from liver RNA and protein (2), only the binding to liver DNA was examined in other studies (3, 4, 5, 6). Ethyl carbamate produced more liver tumors in male than female mice (6). Orchidectomy reduced the tumor-yield and while ovariectomy increased the yield in females it was not to the level of that in intact males (7). The greater hepato-carcinogenicity of ethyl carbamate in males correlated with the greater binding to liver DNA in males although there was no sex difference in the level of binding to DNA of tissues in which there was no sex-difference in tumor yield, i.e. epidermis and lung (6).

The role of the sex-hormones in this difference has been examined in intact, castrated and castrated plus sex-hormone treated mice.

MATERIALS AND METHODS

Male and female Crackenbush mice, 7-8 weeks old at the start of each experiment, were used. Orchidectomy and ovariectomy were performed under light ether anesthesia. Three weeks later, the mice were given four, daily, consecutive intramuscular injections of estradiol monobenzoate (2 x 10 µg. followed by 2 x 2 µg.), testosterone propionate (4 x 10 µg.) (Knoll Laboratories, Sydney, Australia) or the vehicle (peanut oil). Twenty four hours after the last injection, of hormone or vehicle, the mice were given a single intraperitoneal injection (50 µCi; 20 mg.) of ethyl carbamate -(2-^{3}H)(154 mCi m moles $^{-1}$) (Radiochemical Centre, Amersham, G.B.). Mice were killed in groups of eight, 6, 12, 24 and 48 hours later. The livers were rapidly excised and frozen in liquid nitrogen. Liver DNA was extracted by the phenol

technique (8). The specific activity of the DNA was measured in a perchloric acid hydrolysate (70°C for 30 minutes). The DNA content was measured by the diphenylamine technique (9).

RESULTS

The binding of the metabolite, or metabolites, of ethyl carbamate is expressed as nanomoles g. DNA^{-1}. These values were obtained by dividing the radioactivity associated with DNA by the specific activity of the tritiated ethyl carbamate and thence correcting for the total dose of carbamate administered (tritiated plus unlabelled). It is not intended to imply that the whole ethyl carbamate molecule binds, indeed it is known that only the ethyl group, with or without the oxygen atom linking carbon-1 with the carbonyl carbon, binds (1). Some of the results quoted here for intact male and female mice have appeared elsewhere (4,6).

The maximum levels of binding measured in intact and ovariectomised female mice were similar 30.1 and 31.8 n moles g. DNA^{-1} respectively, although the maximum was probably achieved later in the latter group. In the ovariectomised mice, the binding was maintained at a higher level for longer than in intact females. At 24 and 48 hours the levels were 31.8 and 14.1 n moles g. DNA^{-1} compared with 7.2 and 1.3 n moles g. DNA^{-1} in intact females. Testosterone did not alter the level of binding in ovariectomised females. However it must be pointed out that administration of estradiol did not fully restore the levels of binding to those in intact females.

In male mice, orchidectomy resulted in a very much lower level of binding. At 24 hours, the binding in intact males

was 276.3 n moles g. DNA^{-1} compared with 9.8 n moles g. DNA^{-1} in orchidectomised males. In the orchidectomised males, the level of binding did not alter throughout the 48 hours of examination. Administration of testosterone to orchidectomised mice restored the level of binding to that found in intact males whereas the levels in orchidectomy plus estradiol-treated orchidectomised mice were the same as in orchidectomised males.

TABLE I.

Binding to liver DNA after a single dose of ethyl carbamate - (2-^{3}H) (50 μCi; 20 mg.; i.p.)

Sex	Treatment	Binding to liver DNA (nanomoles g. DNA^{-1})			
		6 hr.	12 hr.	24 hr.	48 hr.
M	Intact	27.8	276.3	65.3	55.2
	Castrate	7.1	9.8	6.7	7.2
	Castrate + estradiol	8.1	6.7	12.2	9.1
	Castrate + testosterone	23.4	257	101.6	47.3
F	Intact	19.9	30.1	7.2	1.3
	Castrate	10.9	26.8	31.8	14.1
	Castrate + estradiol	23.5	27.6	17.2	-
	Castrate + testosterone	10.0	35.3	32.5	6.6

DISCUSSION

Many chemicals, including ethyl carbamate, have different carcinogenicities in male and female animals, (8). Ethyl carbamate produced more hepatocellular tumors in male mice although there was no sex difference in the yields of epidermal and lung tumors (6). In a separate study, orchidectomy reduced the hepatocellular tumor yield whereas ovariectomy increased it but only to the level in orchidectomised males (7). The binding of a carcinogen, or a metabolite, to DNA, RNA and/or protein appears to be involved in the carcinogenicity of many chemicals (9). The binding of an ethyl or ethoxy group to DNA correlates with the carcinogenicity of ethyl carbamate in terms of both tissue and sex susceptibility (6).

The present results indicate that the binding of ethyl carbamate metabolites to liver DNA is more sensitive to hormonal changes in male mice. The obvious dependance of binding on androgen levels shown by the affect of testosterone administration to orchidectomised males is substantiated by the enhancement of binding in ovariectomised females, with and without testosterone treatment. In the former group it is thought that the androgen-estrogen balance would move in favor of the androgens by the removal of the major source of estrogens, i.e. the ovaries.

The difference in the levels of binding in male and female mice is highlighted by altering the hormonal status. It is not known why this difference occurs but there are many points in the sequence of events from the administration of ethyl carbamate to the measurement of binding at which the difference could be manifested. Ethyl carbamate requires metabolism, presumably by microsomal enzymes (10) and it is known that many chemicals are metabolised at a greater rate in males (11) although with

the substrates used this does not appear to hold true for mice (12). If the binding to liver DNA can be used as a measure of the metabolism of ethyl carbamate, the results presented here are in accord with the established knowledge on sex differences and the effects of gonadectomy on microsomal enzymes (13). However, as measured by the excretion of CO_2, there was not sufficient difference in the rates of metabolism to account for the much greater binding in males. The possibility exists that the production of an alkylating agent, the ethyl or ethoxy group, from ethyl carbamate represents an anomolous aspect of the metabolism.

Alternative hypotheses must involve differences in the DNA. Differences in DNA content are known, there being a greater degree of polyploidy in male rats (14). The lower level of binding in females could be due to a greater rate of removal of bound material from the DNA.

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