Effect of 3-Methylcholanthrene on Thyroid Function in Sprague-Dawley Rats

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Summary

Fifty-day-old Sprague-Dawley females received daily feedings of 10 mg of 3-methylcholanthrene (MCA) in sesame oil for 30 days. Controls received only sesame oil. Thyroid activity was estimated using the thyroid secretion rate (TSR) method. TSR of each rat was determined prior to, during, and after MCA treatment. Average TSR of sesame oil controls did not change either during or after feeding. Average TSR of MCA-treated rats decreased approximately 25% during the feeding period when compared to the average TSR obtained prior to the MCA treatment. Average TSR after MCA treatment was comparable to that obtained before administration of the carcinogen. The rate of thyroidal 131I release was also determined in MCA-treated rats. Beginning 24 hr after the administration of 131I, each rat received daily intragastric feedings of 1 ml of sesame oil for 3 days, followed by daily feedings of 10 mg of MCA in 1 ml of sesame oil for another 5-7 days. Average hourly release of thyroidal 131I was significantly less during the MCA feeding period than during the control period in which the rats received only sesame oil. Average hourly release of thyroidal 131I of diet-restricted animals was reduced to approximately the same degree as that of MCA-treated rats. However, the average plasma PBI concentration of MCA-treated rats was significantly less than that of diet-restricted rats. These data suggest that the effects of MCA administration on thyroid function may be partially, but not entirely, due to decreased food intake by the treated animals.

Introduction

Although the incidence of spontaneous mammary cancer in Sprague-Dawley rats is relatively low (13, 20), a high incidence of mammary cancer can be induced in these animals by several chemical carcinogens (12). For example, the daily administration of 10 mg of MCA for 20-30 days results in mammary cancer in all animals so treated (11). The incidence, latent period, and growth rate of mammary cancer induced with this carcinogen have been shown to vary with dosage, route of administration, age, and the endocrine status of the host (7, 12). Most of the MCA-induced mammary cancers are hormone responsive to hormones results in regression of established tumors or retardation of tumor induction rate (7, 11, 12).

Little is known, however, concerning the effect of the carcinogen per se upon endocrine function in the recipient animal. Recently, we have shown that MCA administration results in a release of prolactin from the adenohypophysis (16), whereas other studies (14) suggest that MCA may inhibit secretion of the other pituitary gonadotrophins. Since several investigations have indicated an apparent relationship between thyroid function and mammary cancer (2, 3, 8, 10, 15, 21), we became interested in determining whether MCA administration resulted in an alteration of thyroid function.

Materials and Methods

Young, sexually mature, female Sprague-Dawley rats were kept in a room which was artificially illuminated during daylight hours and maintained at a temperature of 76 ± 2°F. Food and water were available ad libitum. Thyroidal radioactivity was measured with a lead-shielded scintillation detector (Harschel model 8SF8) containing a 2-inch thallium-activated sodium iodide crystal. The detector was in turn connected to a Nuclear Chicago model 183 B scaling unit. External neck counts were taken at regular intervals. Each animal was lightly anesthetized with ether and placed in a prone position upon a 2-inch thick lead brick with the ventral region of the neck exposed to the scintillation probe via a 1-inch circular opening in the brick. Care was taken in placement of the animal to ensure the same geometric relationship at each successive counting period. Conventional corrections were made for background radiation and decay of the isotope.

Determination of Thyroid Release Rate. Rats were placed on a low iodine diet (Remington) 7 days prior to the administration of 131I to assure a high uptake of radioactive 131I by the thyroid gland. At age 50 days, each rat received either 0.5 or 1.0 ^c of carrier-free 131I by i.p. injection. Twenty-four hr later an initial neck count was taken and the low iodine diet was replaced by Wayne Lab Blox. Beginning on the day of the initial neck count, each rat received daily intragastric feedings of 1 ml of sesame oil for 3 days, followed by daily feedings of 10 mg of MCA in 1 ml of sesame oil for another 5-7 days. Daily neck counts were taken during the entire treatment period. Thyroidal (neck) radioactivity, expressed as the average % injected dose, was plotted against time in days on semilogarithmic paper. Regression lines were calculated separately from the neck counts made during sesame oil feeding and during MCA feeding. The
techniques and terminology of Brownell (5) were used in analyzing thyroidal 131I release curves. The rate constant, designated $k'_4$, for the effective hourly release of thyroidal 131I was calculated from the slope of each regression line. The experimentally determined rate constant, $k'_4$, serves as an estimate of the true rate constant ($k_4$) for the release of thyroid hormone, but is less than $k_4$ as a result of thyroidal reutilization of 131I derived from the metabolic degradation of endogenously labeled thyroid hormone (5). Pipes et al. (17) suggested that a more accurate estimate of the true rate of release of thyroid hormone could be obtained by determining thyroidal 131I release rates in animals treated with a goitrogen to block reutilization of 131I. These authors applied the term $k''_4$, to the rate constant for thyroidal 131I release during goitrogen administration. The method proposed by Pipes et al. was employed in a 2nd investigation of the effect of MCA on thyroidal 131I release rate. A group of 10 rats received the same treatment as that described above except that after the initial neck count a 0.0125% solution of methimazole was substituted for their drinking water in order to prevent thyroidal reutilization of 131I.

In another experiment, thyroidal 131I release rates of carcinogen-treated rats were compared to those of a group in which dietary intake was restricted. Animals in the MCA-treated group were placed in individual metabolic cages immediately after receiving 131I. Thereafter, treatment of these animals was identical to that in the experiment described above, wherein methimazole was not administered. The food consumed by each animal was measured daily. Beginning 1 day after the initiation of this regimen, the diet-restricted animals were similarly housed and treated with the exception that the diet-restricted rats received daily intragastric feedings of only 1 ml of sesame oil throughout the experiment. Each rat in the diet-restricted group was randomly paired with a member of the MCA-treated group and was allowed access to only that amount of food consumed by the MCA member of the pair on the preceding day. Thus, the food available to the diet-restricted animals was limited to an amount equivalent to that consumed by the carcinogen-treated animals at a comparable stage in the experiment.

Protein-bound iodine (PBI) concentrations were determined in plasma obtained from the animals which had been used in the experiment comparing the effects of MCA and dietary restriction on the release of thyroidal 131I. Immediately after the final neck counts were made, the animals were anesthetized with ether and a blood sample was obtained from the inferior vena cava of each rat. Plasma was separated by centrifugation, and determination of the plasma PBI concentration of each sample was made by a modification of Barker et al.'s. (1) method.

**DETERMINATION OF THYROID SECRETION RATE.** The TSR of each rat was determined by the thyroxine replacement technic prior to, during, and after MCA treatment. Two $\mu$g of 131I were administered to each rat by i.p. injection at age 38 days and an initial neck count was taken 48 hr later. Each rat received a s.c. injection of 0.25 $\mu$g of L-thyroxine/100 gm/day for 2 consecutive days beginning on the day of the initial neck count. The dose of thyroxine was increased at 0.25 $\mu$g/100-gm increments, each dose being injected for 2 consecutive days. Neck counts were made on the day of each increase in thyroxine dosage. The thyroxine dose which prevented further thyroidal 131I output (95–100% of the previous count) was estimated as the TSR for that animal. Beginning at age 50 days, each rat received daily intragastric feedings of 10 mg of MCA in 1 ml of sesame oil for 30 days. At age 60 days, the animals were again injected with 131I and the TSR determined. Ten days after the MCA feedings were discontinued, at age 90 days, the animals were again injected with 131I and the final TSR was estimated. Control rats of the same age were treated similarly, except that each rat received 1 ml of sesame oil daily for 30 days instead of the MCA solution.

**Results**

The effect of MCA administration on the release of thyroidal 131I is shown in Chart 1. Obviously, the regression lines calculated separately from the neck counts made during the control period (daily sesame oil feedings) and during the experimental period (daily MCA feedings) are different. Extrapolation of each regression line to time zero reveals that the biologic half-life ($t_1/2$) of thyroidal 131I during the control period was approximately 3.5 days, while during MCA treatment the $t_1/2$ was approximately 7 days. These estimates indicate that the rate of release of thyroidal 131I decreased by almost 50% during the MCA treatment period. That such is the case can be seen in Table 1, which compares rate constants ($k''_4$) calculated from the data obtained during each treatment period. The apparent average hourly release of thyroidal 131I during MCA feeding was significantly less than that observed during the control period ($P < .001$). Qualitatively, MCA exerted the same effect upon thyroidal 131I release in methimazole-treated rats as it did in those which did not receive the goitrogen, although goitrogen administration resulted in a more rapid rate of release of thyroidal 131I during both phases of the experiment. The $t_1/2$ of thyroidal 131I during sesame oil and MCA feeding were, respectively, 2.2 and 3.8 days. The average $k''_4$ during MCA feeding was significantly less than that during the oil control period (Table 2).

![Chart 1](chart1.png)

**Chart 1.** The effect of MCA on thyroidal 131I release in rats which were or were not treated with methimazole to block recycling of 131I. Upper curve, no methimazole; lower curve, 0.0125% methimazole solution instead of drinking water.
TABLE 1
THE EFFECT OF S-METHYLCHOLANTHRENE (MCA) ON THE APPARENT RATE OF RELEASE OF THYROIDAL 131I IN RATS

<table>
<thead>
<tr>
<th>Treatment period</th>
<th>$k'4^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1 ml of sesame oil/rat/day)</td>
<td>0.00822 ± 0.00059$^b$</td>
</tr>
<tr>
<td>Experimental (10 mg of MCA/rat/day)</td>
<td>0.00418 ± 0.00021$^c$</td>
</tr>
</tbody>
</table>

*a Observed rate constant for the average hourly release of thyroidal 131I.

*b Mean $k'4 ± S.D.

$c For difference between control and experimental, $P < 0.001$.

TABLE 2
THE EFFECT OF S-METHYLCHOLANTHRENE (MCA) ON THE APPARENT RATE OF RELEASE OF THYROIDAL 131I IN RATS TREATED WITH METHIMAZOLE

<table>
<thead>
<tr>
<th>Treatment period</th>
<th>$k'4^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1 ml of sesame oil/rat/day)</td>
<td>0.01172 ± 0.00077$^b$</td>
</tr>
<tr>
<td>Experimental (10 mg of MCA/rat/day)</td>
<td>0.00513 ± 0.00072$^c$</td>
</tr>
</tbody>
</table>

*a Observed rate constant for the average hourly release of thyroidal 131I when thyroidal reutilization of 131I is prevented by methimazole administration.

*b Mean $k'4 ± S.D.

$c For difference between control and experimental, $P < 0.001$.

TABLE 3
THE EFFECT OF MCA ON THE THYROID SECRETION RATE OF RATS

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of rats</th>
<th>Average TSR (μg T₄-thyroxine/100 gm/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sesame oil (1 ml/rat/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before (38)$^b$</td>
<td>13</td>
<td>0.78</td>
</tr>
<tr>
<td>During (60)</td>
<td>11</td>
<td>0.75</td>
</tr>
<tr>
<td>After (90)</td>
<td>10</td>
<td>0.71</td>
</tr>
<tr>
<td>MCA (10 mg/rat/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before (38)</td>
<td>14</td>
<td>0.88</td>
</tr>
<tr>
<td>During (60)</td>
<td>12</td>
<td>0.65$^c$</td>
</tr>
<tr>
<td>After (90)</td>
<td>7</td>
<td>0.87</td>
</tr>
</tbody>
</table>

*a MCA, 3-methylcholanthrene; TSR, thyroid secretion rate.

$b$ Numbers in parentheses refer to age in days at beginning of TSR determination.

c Significantly different from values obtained before and after treatment ($P < 0.01$).

The estimated average TSR observed during the period of MCA feeding was approximately 25% less than that obtained before MCA treatment (Table 3). The average TSR after MCA feedings were discontinued was comparable to that obtained prior to the administration of the carcinogen. No significant change in the average TSR was evident in rats treated daily with 1 ml of sesame oil for 30 days.

Since the diet-restricted rats always wasted some of the food made available to them each day, the amount of food consumed by these rats on any single day was less than that consumed by their carcinogen-treated partners (Chart 2). The more severe dietary restriction imposed upon these rats resulted in body weights which were significantly less than those of the MCA-treated rats (Chart 2). In Chart 3, it is again demonstrated that the rate of release of thyroidal 131I during the period of MCA feeding is significantly lower than that obtained during the period of oil feeding. However, this same phenomenon was observed in the diet-restricted group, which did not receive MCA, during comparable phases of the experiment. Average $k'4$ of both the MCA-treated and diet-restricted animals was significantly lower during the experimental period (days 6-11) than during control days 1-5 (Table 4). The average plasma PBI concentration of rats which received 6 daily feedings of MCA was approximately 28% less than that of diet-restricted control rats (Table 5). Although the ranges of individual values for plasma PBI concentration in the 2 groups overlap, the average value for the control group was significantly greater than that for MCA-treated rats.
Discussion

Several investigations have indicated that the experimental alteration of thyroid function either by thyroidectomy or the administration of thyroid-active compounds results in a modification of the course of development of chemically induced mammary tumors. Thus, Bielschowsky and Hall (3) have shown that, in the rat, thyroidectomy exerts an inhibitory effect upon the induction of mammary tumors with 2-acetylaminofluorene. Similar results were obtained by Jull and Huggins (15) in the rat, thyroidectomy exerts an inhibitory effect upon the induction of mammary tumors with 2-acetylaminofluorene. Other studies indicate that rats and mice maintained in a hypothyroid state by the administration of goitrogenic drugs also exhibit a reduction in the mammary tumor incidence. Helfenstein et al. (10) reported a 50% reduction in incidence and a delay in appearance of mammary tumors induced with 9,10-dimethyl-1,2-benzanthracene in rats maintained on propylthiouracil throughout the experimental period. Furthermore, the development of spontaneous mammary tumors in several susceptible strains of mice has been prevented or markedly reduced by the administration of thiourea (21) or thiouracil (8). Thus, it is apparent that an alteration in thyroid activity is effective in modifying the tumorigenic response of rats and mice to the chemical carcinogens.

In the present study, decreases in thyroidal 131I release rate and thyroid secretion rate in response to MCA administration clearly indicate that this carcinogenic agent depresses thyroid activity. The reduction in thyroid function effected by MCA, however, appears to be transient for the TSR returns to the control level within a short time after cessation of MCA feedings. The precise mechanism by which this depression occurs is unknown. However, inanition per se is known to depress thyroid activity (18, 19) and our data (Table 4) from animals on a restricted diet confirm this observation. Thus, dietary restriction, whether due to decreased appetite resulting from MCA administration or imposed by experimental design, can at least partially account for thyroid depression under the conditions of these experiments. However, careful measurement revealed that the food consumption of the diet-restricted rats was consistently less than that of the MCA-treated rats. This factor could be of some importance in assessing thyroid function. Furthermore, in rats treated with MCA the average plasma PHI concentration was significantly less than that of the diet-restricted control animals (Table 5). These data suggest that the effects of MCA administration upon thyroid function are not entirely due to decreased food intake by the treated animals. Regardless of the mechanism by which thyroid depression occurs, it is readily apparent that thyroid function is reduced during the period of MCA feeding.

Dao et al. (6) and Flesher and Sydnor (9) have provided evidence that mammary epithelial and adipose tissue levels of MCA are maintained for a considerable period of time after the administration of the carcinogen. Furthermore, Bolanys et al. (4) have indicated that after the oral administration of MCA fat cells surrounding the mammary epithelium were laden with the carcinogen. Although the influence of the degree of thyroid activity on the concentration and/or clearance of MCA at the mammary level has not been investigated, it is possible that a hypothyroid state may serve to increase the retention of the carcinogen at the mammary level and thereby enhance the initial phase of carcinogenesis. In support of this view, Bather and Franks (2) have

### Table 4

The Effects of 3-Methylcholanthrene (MCA) and Dietary Restriction on the Apparent Rate of Release of Thyroidal 131I in Rats

<table>
<thead>
<tr>
<th>Treatment Period</th>
<th>k/μt</th>
<th>MCA^a (food ad libitum)</th>
<th>Sesame oil^c (diet restricted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Days 1-5)</td>
<td>0.00502 ± 0.00029</td>
<td>0.00472 ± 0.00020</td>
<td></td>
</tr>
<tr>
<td>Experimental (Days 6-11)</td>
<td>0.00328 ± 0.00011</td>
<td>0.00319 ± 0.00015</td>
<td></td>
</tr>
</tbody>
</table>

^a Observed rate constant for the average hourly release of thyroidal 131I.
^b Daily intragastric feedings of 1 ml of sesame oil for 10 days.
^c For difference between experimental and control, P < 0.05.

### Table 5

Average Plasma Protein-bound Iodine (PBI) Concentrations in Terminal Blood Samples of 3-Methylcholanthrene (MCA)-treated and Diet-restricted Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PBI (μg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCA^a</td>
<td>3.4 (1.3-4.8)ᵇ</td>
</tr>
<tr>
<td>Restricted diet^c</td>
<td>4.7ᵇ (3.6-5.4)</td>
</tr>
</tbody>
</table>

^a Daily intragastric feedings of 1 ml of sesame oil for 10 days.
ᵇ Range.
^c Mean ± S.D.
^d For difference between MCA-treated and diet-restricted rats, P < 0.05.

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reported that the incidence of tumors at the site of injection of chemical carcinogens is reduced by a single injection of thyroxine and that the rate of disappearance of carcinogens from mice is significantly increased by thyroxine treatment. The question as to whether or not a hypothyroid state may, on the one hand, enhance while on the other, inhibit carcinogenesis apparently depends upon whether hypothyroidism is induced during the initiation or promotion phase of the carcinogenic process. Since MCA-induced hypothyroidism is but temporary, it is probable that growth of MCA-induced neoplastic tissue would be unaffected. Irrespective of whether the degree of thyroid activity proves to be of importance in the initiation of neoplastic change, the effect of the carcinogen (MCA) per se on hormonal secretion is becoming increasingly apparent.

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