**Inhibitory Effect of Hypothalamic Lesions on Liver Tumor Induction by N-2-Fluorenylacetamide in Male Rats**

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**ABSTRACT**

Bilateral electrolytic lesions were placed in the median eminence area of the hypothalamus in 12-week-old male Wistar rats. Sham-operated and untreated control rats were also included. Two weeks later, one-half of them were given 0.03% N-2-fluorenylacetamide incorporated into the diet for 16 weeks with adequate resting periods in between. The animals were killed 34 weeks after the last carcinogen feeding. The results show that lesions in the hypothalamus effectively inhibited liver tumor formation (0 of 16, 0%). In contrast, the incidence of hepatocellular carcinomas in sham-operated rats was 38.5% (5 of 13), and that of untreated controls was 42.9% (6 of 14). No tumors developed in rats fed diet without carcinogen. Testicular atrophy, inactive thyroid glands, and shorter nasoanal lengths were observed in rats with lesions in the hypothalamus irrespective of carcinogen treatment. It is apparent from these data that lesions in the median eminence area of the hypothalamus inhibit the induction of liver carcinogenesis with N-2-fluorenylacetamide in male rats.

**INTRODUCTION**

The incidence of primary liver cancer has been reported to be higher in men than in women (2, 22, 23, 27). A greater male susceptibility to the induction of liver cancer by N-2-fluorenylacetamide or N-hydroxy-N-2-fluorenylacetamide was also found in various strains of rats such as Fischer (13, 58, 59), Wistar (4, 26), Sprague-Dawley (51), Holtzman (31, 32), Buffalo (47, 59), hooded (49), and AXC (35, 41, 59). Castration decreased tumor incidence (12, 35, 42), and administration of testosterone restored the susceptibility (12, 35). Recently, exogenous androgens have also been reported to be associated with the development of hepatocellular carcinoma in humans following long-term treatment of aplastic or Fanconi's anemia with androgenic-anabolic steroids, mainly oxymetholone or methyltestosterone derivatives (30). However, in rats, testosterone was reported to have no direct effect but to act indirectly via other endocrine glands to increase chemical hepatocarcinogenesis (54). The possible mechanism of the action of testosterone on liver tumorigenesis has been thoroughly discussed, and a hypothesis was put forward that the action of testosterone on hepatocarcinogenesis might mediate through the thyroid, the pituitary, and possibly the hypothalamus (55). The contributions of endocrinology to cancer are many, but little has been published on the neuroendocrinological aspect of carcinogenesis. The importance of the hypothalamus in carcinogenesis has been reviewed (56). However, investigations are required to ascertain the role of the hypothalamus in the formation of liver tumors before a precise mechanism of the action of testosterone can be discussed. This report describes the effect of lesions in the median eminence area of the hypothalamus on liver tumor induction by N-2-fluorenylacetamide in male rats.

**MATERIALS AND METHODS**

Male albino Wistar rats, 12 weeks old [body weight, 265.4 ± 3.3 g (S.E.),] were used in this investigation. They were housed in wire-bottomed cages in groups of 4. The average temperature of the room was 29.7 ± 0.2° (S.E.) with about 12 hr/day of light. The animals were given a constant pelleted and tap water ad libitum.

The hypothalamic lesions were placed in the median eminence area of the hypothalamus with the use of Stoeoting stereotaxic instrument and with the aid of de Groot's (10) atlas of the rat brain. The electrodes were constructed from 24-gauge Nichrome wire (VWR Scientific Catalog No. 66258-124). They were cleaned with ether, insulated with 5 coatings of varnish, and dried between each coat for 24 hr in a 100° oven. The tips of the insulated electrodes were ground flat, and the insulation was tested with a volt-ohmmeter. The median eminence lesions were produced bilaterally by passing a direct current of 4 ma through the electrodes for 15 sec. The operations were performed on rats anesthetized with a small i.v. dose of pentobarbital and maintained with ether during the stereotaxic procedures. Sham lesions were created in rats by lowering the electrode at the identical coordinates without passing electrical current. Untreated control rats were also included. The rats were randomly allocated into various experimental groups. Two weeks later, one-half of them were given 0.03% N-2-fluorenylacetamide incorporated into the diet. The carcinogen-containing diet was ingested for 4-week periods followed by 1 week on diet without carcinogen until the carcinogen had been administered for 16 weeks. The rats were then given laboratory pellets for the remainder of their life.

The animals were killed 34 weeks after the last carcinogen feeding. The brains were removed, cut in serial sections at 10 μm, mounted on 35-mm leader films, stained with cresyl violet and Luxol fast blue, and covered with a hardening plastic spray. The sections were examined under low-power microscopy, and the location of the lesions was confirmed (Fig. 16). The liver, kidneys, adrenal glands, thyroid glands, pituitary glands, and other organs that showed any abnor-
malities were removed routinely for histological examination. The liver lesions were classified according to the recent report of a workshop on classification of specific hepatocellular lesions in rats (50). Statistical significance was calculated by Student's t test.

**RESULTS**

With the exception of some of the hypothalamus-lesioned rats, rats that ate normal diet throughout the experiment had completely normal livers. The liver cells of some of the rats with lesions in the median eminence area of the hypothalamus, regardless of whether the rats were fed diet with or without carcinogen, contained fat droplets (Fig. 1), and the cells in the region of the central veins were so distended with fat that they ruptured. By fusion with adjacent ruptured cells, fatty cysts were formed. Fatty liver has also been reported in some of the rats (7), in dogs (16), and in 2 cases in humans (34) with hypothalamic lesions.

The incidence of hepatic lesions in various groups of rats treated with carcinogen is shown in Table 1. No liver tumors developed in rats with lesions in the median eminence area of the hypothalamus, although 3 of 16 rats showed foci of cellular alteration. In contrast, the incidence of hepatocellular carcinomas in sham-lesioned rats was 38.5% (5 of 13) and that of untreated control rats was 42.9% (6 of 14). Five of the hepatocellular carcinomas had poorly or well-differentiated trabecular types (Figs. 4 and 5). Only 2 rats, 1 in the sham-lesioned group and 1 in the untreated control group, showed areas of cholangiofibrosis. Carcinogen increased liver weights in both the untreated control and the sham-lesioned groups but not in the rats with hypothalamic lesions.

Body weights were not significantly different among the control and experimental groups except for sham-lesioned rats treated with carcinogen; these rats have a lighter body weight than do non-carcinogen-treated experimental rats and untreated control rats. However, the sham-lesioned rats were not significantly different statistically from those with lesions in the median eminence area of the hypothalamus. Carcinogen increased liver weights in both the untreated control and the sham-lesioned groups but not in the rats with hypothalamic lesions (Table 2). The liver

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of animals</th>
<th>No. of areas of cellular alteration</th>
<th>Neoplastic nodules</th>
<th>Hepatocellular carcinomas</th>
<th>Cholangiofibrosis (adenofibrosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>14</td>
<td>2 (14.3)</td>
<td>6 (42.9)</td>
<td>1 (7.1)</td>
<td></td>
</tr>
<tr>
<td>Hypothalamic lesions</td>
<td>16</td>
<td>3 (19)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sham lesions</td>
<td>13</td>
<td>2 (15.4)</td>
<td>1 (7.7)</td>
<td>5 (38.5)</td>
<td>1 (7.7)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of animals.

### Table 2

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Liver weight (g)</th>
<th>Brain weight (g)</th>
<th>Final body weight (g)</th>
<th>Body length (mm)</th>
<th>Seminal vesicles (mg)</th>
<th>Testes (mg)</th>
<th>Kidneys (mg)</th>
<th>Spleen (mg)</th>
<th>Nasal length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>12</td>
<td>K</td>
<td>364.7 ± 14.90 §</td>
<td>3.3 ± 0.16</td>
<td>3.97 ± 1.0 ± 121.14 = 1.45</td>
<td>0.33 ± 0.02</td>
<td>3.77 ± 186.66 ± 15.55</td>
<td>0.68 ± 0.02</td>
<td>0.19 ± 0.01 22.86 ± 0.22</td>
</tr>
<tr>
<td>Sham lesions</td>
<td>10</td>
<td>N</td>
<td>346.44 ± 17.16</td>
<td>5.55 ± 0.71</td>
<td>3.53 ± 0.21 ± 14.01 = 1.36</td>
<td>0.84 ± 0.02</td>
<td>169.26 ± 20.50 ± 164.30</td>
<td>1.50 ± 0.39</td>
<td>0.72 ± 0.04 22.96 ± 0.23</td>
</tr>
<tr>
<td>Hypothalamic lesions</td>
<td>11</td>
<td>K</td>
<td>340.87 ± 31.99</td>
<td>2.98 ± 0.12</td>
<td>4.98 ± 0.72 ± 14.43 = 1.50</td>
<td>0.83 ± 0.02</td>
<td>69.58 ± 15.80 ± 18.90</td>
<td>0.47 ± 0.03</td>
<td>0.72 ± 0.04 21.46 ± 0.39</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Liver weight (g)</th>
<th>Brain weight (g)</th>
<th>Final body weight (g)</th>
<th>Body length (mm)</th>
<th>Seminal vesicles (mg)</th>
<th>Testes (mg)</th>
<th>Kidneys (mg)</th>
<th>Spleen (mg)</th>
<th>Nasal length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>13</td>
<td>K</td>
<td>317.32 ± 25.20</td>
<td>2.95 ± 0.16</td>
<td>4.76 ± 0.30 ± 16.44 = 1.81</td>
<td>0.48 ± 0.05</td>
<td>74.69 ± 19.57 ± 19.40</td>
<td>0.63 ± 0.04</td>
<td>0.22 ± 0.03 21.70 ± 0.21</td>
</tr>
<tr>
<td>Sham lesions</td>
<td>10</td>
<td>N</td>
<td>343.76 ± 25.66</td>
<td>3.24 ± 0.19</td>
<td>4.25 ± 0.28 ± 13.39 = 1.34</td>
<td>0.76 ± 0.04</td>
<td>125.03 ± 16.03 ± 16.30</td>
<td>0.63 ± 0.04</td>
<td>0.22 ± 0.03 22.56 ± 0.18</td>
</tr>
<tr>
<td>Hypothalamic lesions</td>
<td>14</td>
<td>K</td>
<td>257.15 ± 16.74</td>
<td>4.55 ± 0.38</td>
<td>5.50 ± 0.59 ± 19.03 = 1.61</td>
<td>0.74 ± 0.04</td>
<td>84.65 ± 14.50 ± 14.26</td>
<td>0.64 ± 0.03</td>
<td>0.30 ± 0.03 22.54 ± 0.19</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of animals.
weights of both groups of rats with hypothalamic lesions were quite similar regardless of whether or not they were treated with carcinogen. As compared to the untreated controls and sham-operated rats, lesions in the median eminence area of the hypothalamus greatly reduced the weights of the testes and seminal vesicles. Histologically, testicular deficiency involved failures of interstitial cells of Leydig and germinal cells (Fig. 10). Spermatogenesis arrested at one of the immature stages. Spermatozoa and spermatids were absent or rare, and many seminiferous tubules were lined with Sertoli’s cells and spermatogonia alone. The seminal vesicles became atrophie (Fig. 12).

Although thyroid gland weights were not statistically significantly different between controls and lesioned rats, there were changes in thyroid histology (Fig. 15). The thyroid glands of hypothalamus-lesioned rats irrespective of carcinogen treatment were inactive, with abundant colloid and large follicles, and the cells lining them were flat compared to normal cells. The adrenal glands remained histologically normal. The total lengths of the body measured from the nose to the anus were significantly shorter in rats with lesions in the median eminence area of the hypothalamus regardless of whether or not they were treated with carcinogen. The pituitary stalk and gland were intact on autopsy but were lost during storage as a result of electrical power failure; therefore no weights can be given for these. Lung abnormalities were observed in various groups with considerably more in sham- and hypothalamus-lesioned rats than in the untreated controls irrespective of carcinogen treatment. Two mammary gland tumors were found in rats fed N-2-fluorenylacetamide, one in the untreated control and the other in the hypothalamus-lesioned group. Ear duct tumors appeared in 2 of the untreated controls, and 1 ear duct tumor appeared in the sham-operated rats treated with carcinogen.

**DISCUSSION**

It was apparent from this study that hormones of the hypothalamus (now called hypophysiotropic hormones or releasing factors) were involved in the induction of liver tumors by the chemical carcinogen. Lesions in the median eminence area of the hypothalamus effectively inhibited the formation of hepatocellular carcinoma in rats fed diets containing 0.03% N-2-fluorenylacetamide. Hypothalamic lesions that affect pituitary function in the rat are located in the median eminence. Unfortunately, the pituitary was not available for evaluation in animals in which hypothalamic lesions were induced.

In order to clarify whether the hypothalamic lesions on hepatocarcinogenesis were working independently or through the pituitary gland, an experiment was therefore conducted. Fig. 18 shows that, in rats, lesions in the median eminence area of the hypothalamus decreased the population of pituitary cells that had undergone degeneration as compared with the normal ones (Fig. 17). The acidophilic cells lost their acidophilic granules and became indistinguishable in histological preparations from chromophobes.

The pituitary gland, which once was called the master of endocrine glands, now becomes the servant of the hypothalamus, because its functions have been discovered to be controlled by the central nervous system. The hypothalamus is the final common pathway through which various levels of nervous stimuli can affect the function of the pituitary. One may speak of the median eminence as a “gland” in which are located nerve terminals that secrete hypophysiotropic hormones. Lesions in the median eminence area of the hypothalamus inhibit the release of all anterior pituitary hormone-releasing or inhibitory factors. These in turn affect the pituitary target endocrine organs (for a general review of this topic, see Refs. 28 and 53). In this investigation, atrophies of endocrine organs (except adrenal glands) that are targets of the pituitary were observed in hypothalamus-lesioned rats irrespective of carcinogen treatment.

Thyroid hormones were required for hepatocarcinogenesis because thyroidectomy completely suppressed liver tumor formation in rats fed N-2-fluorenylacetamide (6, 38). This does not mean, however, that the inhibitory effect of hypothalamic lesions on hepatic tumorigenesis was directly due to the atrophy of the thyroid gland because, even in the presence of intact thyroid gland and normal hypothalamus, hypophysectomy was reported to inhibit totally the hepatic carcinogenicity of several powerful carcinogens of azo dye, aflatoxin, and aminofluorene groups (15, 18, 36, 48). Administration of thyroid hormone in the drinking water to hypophysectomized rats with intact normal hypothalamus also failed to produce any signs of hepatic carcinogen effect (5). In addition, hypophysectomy of rats 14 weeks (48) but not 23 weeks (40) after the initiation of carcinogenesis completely prevented the conversion of hyperplastic liver cells into malignant tumors, whereas thyroidectomy after 14 weeks (6) or 23 weeks (40) of carcinogen feeding did not totally inhibit tumor development. Even after 23 weeks of carcinogen administration, the degree of protection against liver tumor formation was still greater in hypophysectomized rats than in thyroidec- tomized ones (percentages of hepatic carcinoma formation: untreated control, 10 of 12, 83%; thyroidectomy, 7 of 14, 50%; and hypophysectomy, 3 of 12, 25%) (40). Therefore, inhibition of liver cancer induction by N-2-fluorenylacetamide in hypothalamus-lesioned rats seems more likely to be a consequence of secondary induction of pituitary deficiency rather than thyroidal atrophy. Of course, pituitary damage would affect thyroid function. In this experiment, although thyroid gland weights were not significantly different between control and experimental rats, histological evidence of thyroidal atrophy were prominent in animals with lesions in the median eminence area of the hypothalamus. This indicates that there was suppression of thyrotropic hormone release from the adenohypophysis. Median eminence destruction-produced thyroidal atrophy has also been observed in the dog (14).

If the effect of the hypothalamus on hepatic carcinogenesis was mediated via the pituitary gland, then the question arises as to which of the pituitary hormones is more important in the modulation of the carcinogenic process in the liver. Bielschowsky and Hall (6) reported that failure to develop liver tumors in thyroidec- tomized rats was due to the absence or decreased number of pituitary acidophils.
and therefore decreased the level of GH secretion that would normally be required for the tumor development. A good correlation between prompt acidophil degranulation and the low level of GH has been shown (45). Recently, a group of Japanese workers (19, 24, 60-62), basing their findings on histochemical and electron microscopic studies, reported that in rat pituitary there were 2 types of acidophilic granules: the large ones (approximately 350 nm) responsible for GH secretion and the small ones (about 130 nm) responsible for adrenocorticotropic hormone release. Thyroidectomy decreased the number of large acidophilic granules but had no remarkable effect on the small ones, and injection of thyroxine returned to normal. In rats radioimmunoassay of both pituitary and plasma GH showed a fall following thyroidectomy and an increase with thyroxine replacement (20, 21). A marked reduction in the rate of amino acid incorporation into the pituitary GH was evident after thyroidectomy, and administration of thyroxine to the thyroidectomized rats restored synthesis (1, 52). Injection of synthetic TRH i.v. resulted in a significant and dose-related increase in plasma GH in the urethane-anesthetized rat (25), and its action was shown to be directly on the anterior pituitary (57). TRH injection also raised plasma GH in rats subjected to hypothalamic ablation (8). In fact, a hypophysis-mediated action of thyroxine on body growth was clearly demonstrated in rats by Earley and Leblond (11) and Scow (46).

If GH is the main important anterior pituitary hormone responsible for liver tumor induction by chemical carcinogens, then an increase in the level of growth hormone should increase tumor formation. Indeed, thyroidectomized rats given GH developed N-2-fluorenylacetamide-induced hepatocellular carcinoma (3). In hypophysectomized rats, GH could restore the activity of azo dye in liver tumor (57). TRH injection also raised plasma GH in rats subjected to hypothalamic ablation (8). In fact, a hypophysis-mediated action of thyroxine on body growth was clearly demonstrated in rats by Earley and Leblond (11) and Scow (46).

The adrenal glands seem not to play an important role in hepatocarcinogenesis. Adrenalectomy did not prevent the development of hepatocellular carcinoma in rats ingesting N-2-fluorenylacetamide (37). Administration of cortisone produced no appreciable effect (12, 40), and deoxycorticosterone acetate even decreased liver tumor incidence (12, 37). In this investigation, there were no differences in mean adrenal weights between control and lesioned rats. Histologically, these adrenal glands remained normal.

Castration of adult male rats were reported to decrease but not completely inhibit liver tumor induction by chemical carcinogen (12, 35, 42, 44). When rats were given GH, the incidence of liver cancer rose in both castrated male rats and ovariectomized female rats (39). In this report, testis weights were significantly reduced in rats with lesions in the median eminence area of the hypothalamus as compared to the normal controls or sham-lesioned group irrespective of carcinogen treatment. Histologically, these testes showed definite atrophy. It seems probable, therefore, that the release of gonadotropic hormones was inhibited in these animals. Testicular atrophy following hypothalamic lesions has been reported previously (9, 29).

The presence of atrophy of the seminal vesicle in hypothy- lamas-lesioned rats indicates the hormone production by the interstitial cells of the testis must also have been reduced.

Since testosterone might be responsible for the higher incidence of liver cancer in males than in females (55) and since testosterone was reported to have no direct effect on liver carcinogenesis (54), there are several ways in which testosterone might act through the thyroid, the pituitary, and possibly the hypothalamus, inasmuch as the present investigation indicates that the hypothalamic did occupy an important role in liver cancer induction. Testosterone might act through the hypothalamus, which in turn acts on the pituitary to increase its secretion, presumably GH, and therefore increase liver tumor incidence, but this mechanism would not be able to account for the complete inhibition of hepatic tumorigenesis after thyroidectomy (6, 38). Possibly, testosterone increases TRH from the hypothalamus and therefore increases pituitary TSH secretion. A higher level of TSH would certainly enhance thyroid hormone secretion, which in turn acts on the pituitary to increase GH release. Alternatively, testosterone may act directly on the thyroid, which then causes an increase of somatotropic hormone-releasing factor from the hypothalamus, therefore increasing pituitary GH secretion. Nevertheless, further investigations are required and it seems that very little attention has been drawn to this aspect of hepatocarcinogenesis. This problem must be solved if a full understanding of the process of carcinogenesis is to be achieved.

**ACKNOWLEDGMENT**

I thank Lim Siew Tiang for histological assistance.

**REFERENCES**

Fig. 1. Fatty liver. Virtually every liver cell contains fat droplets. H & E, × 400.

Fig. 2. Neoplastic nodule consisting predominantly of basophilic cells. H & E, × 20.

Fig. 3. Junction of neoplastic nodule with normal liver. Note the sharp demarcation between the nodule and the surrounding liver cells. H & E, × 200.

Fig. 4. Junction of hepatocellular carcinoma with normal liver. Surrounding liver plates are compressed and tangentially arranged around tumor. H & E, × 400.
Fig. 5. Poorly differentiated hepatocellular carcinoma. Tumor cells are in plates and nests. H & E, x 140.

Fig. 6. Poorly differentiated hepatocellular carcinoma showing large and vesiculate nuclei with prominent nucleoli. H & E, x 400.

Fig. 7. Moderately well- or well-differentiated hepatocellular carcinoma. Tumor cells are individualized or arranged in linear, papillary pattern. H & E, x 200.

Fig. 8. Moderately well- or well-differentiated hepatocellular carcinoma showing hepatocytes in pseudoacinar patterns. Note eosinophilic cytoplasm and hyperchromatic nuclei. H & E, x 400.
Fig. 9. Normal testis. H & E, x 50.
Fig. 10. Testis of hypothalamus-lesioned rat. H & E, x 75.
Fig. 11. Normal seminal vesicle. H & E, x 50.
Fig. 12. Atrophic seminal vesicle of hypothalamus-lesioned rat. H & E, x 50.
Fig. 13. Normal thyroid gland. H & E, × 200.

Fig. 14. Coronal section through rat hypothalamus showing the normal area of median eminence (ME). ARH, arcuatus hypothalami nucleus; VMH, ventromedialis hypothalami nucleus; FX, fornix. Cresyl violet-Luxol fast blue, × 20.

Fig. 15. Thyroid gland of hypothalamus-lesioned rat. Note large follicles with abundant colloid; the cells lining them are flat. H & E, × 200.

Fig. 16. Coronal section through rat hypothalamus showing lesion in the median eminence. Cresyl violet-Luxol fast blue, × 20.
Fig. 17. Anterior pituitary of a normal control rat. H & E, × 1000.

Fig. 18. Anterior pituitary of rat with lesions in the median eminence area of the hypothalamus. H & E, × 1000.
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