Inhibition of Preovulatory Gonadotropin Secretion and Stimulation of Prolactin Secretion by 7,12-Dimethylbenz(a)anthracene in Sprague-Dawley Rats

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ABSTRACT

Serum luteinizing hormone, follicle-stimulating hormone, prolactin, thyroid-stimulating hormone, and growth hormone and hypothalamic luteinizing and thyrotrophin contents were measured at given times of the estrous cycle in a dimethylbenz-(a)anthracene (DMBA)-susceptible strain of rat (Sprague-Dawley) and in a DMBA-resistant strain of rat (Wistar) for periods up to the appearance of the first mammary tumors in DMBA-susceptible animals. Tumors usually appeared with ~100% incidence around the 14th to 15th estrous cycle after DMBA treatment in Sprague-Dawley rats.

Hormonal determinations were done by using groups of 4-day cycling rats of both strains which were given DMBA or the vehicle (sesame oil) on a single diestrus I at around 55 days of age. Animals were sacrificed by decapitation without previous anesthesia on the morning and afternoon of proestrus and estrus during the 5th and 11th estrous cycles after treatment.

In Sprague-Dawley female rats, DMBA significantly inhibited luteinizing hormone and follicle-stimulating hormone surges and stimulated the prolactin surge on the afternoon of proestrus at any estrous cycle after treatment (the timing of preovulatory surges was the same in both strains for any estrous cycle or treatment); no difference was found for any hormone at other times of the estrous cycle. In contrast, Wistar rats did not show deranged preovulatory or basal prolactin and gonadotropin release after treatment with the carcinogen; in addition, no difference was found for any other hormone at any time tested.

These results show that there is a specific and transient hormonal deregulation in a DMBA-susceptive strain of rats. Inasmuch as the hormonal imbalance was essentially the same throughout the induction period, an early and persistent alteration in centers implicated in the hormonal cyclicity of the hypothalamopituitary axis must result from DMBA treatment.

INTRODUCTION

It was well established after the initial observations of Huggins et al. (8) that mammary tumors are induced in the female of a susceptible strain of rat (SD) after administration of a potent mammotrophic carcinogen, DMBA. It was further demonstrated by the same authors that DMBA must be adminis-

ered to animals at a given time of life (between 50 and 60 days of age) to obtain tumors with about 100% efficiency. Subsequently, the involvement of the ovary and pituitary on the induction and growth of DMBA-induced mammary cancers was reported (3, 8, 17). In spite of these endocrine requirements, no study of the estrous cycle and possible alterations in the secretion of pituitary and ovarian hormones during the growth of mammary tumors has been reported. We recently reported in a preliminary note (6) that DMBA treatment permanently alters preovulatory PRL and gonadotropin release in DMBA-susceptible female rats. In the present work, we deal with changes in the length of the estrous cycle and in the serum levels of LH, FSH, PRL, TSH, and GH and in hypothalamic LHRH and TRH after DMBA treatment in DMBA-susceptible (SD) and DMBA-resistant (W) female rats.

MATERIALS AND METHODS

Forty-day-old female SD rats (IFFA-CREDO, Lyon, France) and W rats (our laboratory strain, Gif-sur-Yvette, France) were housed under controlled conditions (22°C; monitored light-dark cycles with lights on from 5:30 a.m. to 7:30 p.m.). They were given food pellets (U.A.R., Versailles, France) and water ad libitum.

Vaginal smears were taken every day, and only rats with regular 4-day estrous cycles were used.

DMBA or the carrier solution was administered without anesthesia by the gastric intubation method of Huggins et al. (8). Each rat received 15 mg of DMBA (Calbiochem-Behring Corp., La Jolla, Calif.) in 1 ml of sesame oil. They were treated in the morning of a diestrous I day between Days 55 and 60 after birth. Control animals of the same age received oil only.

Experiment 1. Eighty SD and 80 W female rats were treated with DMBA as above; 80 other rats of each strain served as controls. Vaginal smears were taken every day up to 90 days after treatment for SD rats and up to 180 days after treatment for W rats. These animals were used to follow cyclicity and to observe the occurrence of mammary tumors. No hormonal determinations were made.

Experiment 2. Forty SD and 40 W rats were treated with DMBA; 40 other rats of each strain served as controls. Five animals of each treatment group and strain were sacrificed by decapitation on proestrus morning (10 a.m.) and afternoon (5 p.m.) and on estrous morning (10 a.m.) and afternoon (5 p.m.) at the 5th and 11th estrous cycles after treatment.

Experiment 3. Fifty SD and 50 W rats were treated with DMBA; 60 other rats of each strain served as controls. Groups of 5 animals corresponding to each treatment and strain were sacrificed at 1, 4, 5, 6, and 7 p.m. on the proestrus day of the 5th and 11th cycles following DMBA or oil treatment.
Hormone Assays. The samples of serum LH, FSH, and PRL were assayed in duplicate using the previously described RIA's (11, 12). Serum TSH and GH were assayed using the reagents provided by the National Institute of Arthritis, Metabolism, and Digestive Diseases. All results were expressed as ng per ml in terms of: (a) a laboratory rat LH preparation (1.4 × NIH LH S1); (b) a laboratory rat FSH preparation (30 × NIH FSH S1); and (c) National Institute of Arthritis, Metabolism, and Digestive Diseases rat PRL, TSH, and GH RP-1. The sensitivities of the RIA's for serum LH, FSH, PRL, TSH, and GH assays were 0.09, 0.7, 10, 1, and 2 ng/ml, respectively. The samples of hypothalamic extracts were assayed for LHRH and TRH using the previously described RIA's (10) in duplicate at 2 dilutions, and LHRH and TRH were expressed in terms of synthetic preparations (ng/hypothalamus) provided by Drs. Studer and Gillessen (Hoffman-LaRoche Inc., Basel, Switzerland).

Hormones were assayed in 2 separate groups, corresponding to Experiments 2 and 3. The interassay variabilities for LH, FSH, PRL, TSH, LHRH, and TRH were 9, 15, 12, 5, 7, 5, and 7% respectively.

Data Analysis. For statistical analysis, the Student's t test and the Fisher-Snedecor F test were used. A p value of 0.05 or less was considered significant.

RESULTS

Effect of DMBA or Oil Treatment on the Regularity of the Estrous Cycle in SD and W Rats

It can be seen from Table 1 that the estrous cycles of animals of both strains were affected by any treatment. Regardless of the term of treatment (short or long) or the type of treatment (DMBA or oil) of either SD or W rats, the alteration consisted of the lengthening of the estrous cycle. In short-term groups (up to the 3rd estrous cycle after treatment), 5- or 6-day estrous cycles were observed with an additional 1 or 2 days of estrus. In the long-term groups (between the 6th and the 9th estrous cycle after treatment), a blockade at diestrus was observed for 3 to 7 days before the animals returned to a 4-day estrous cycle.

In the short-term group, the alteration of the estrous cycle, which was essentially the same for both treatments, was much more pronounced in SD than in W rats. However, with long-term treatment, a lengthening of the estrous cycle was observed only in SD rats treated with DMBA. Interestingly, those W rats which developed mammary tumors also exhibited a lengthening of the estrous cycle at the same period.

Effect of DMBA Treatment on Serum Levels of Pituitary Hormones and on Hypothalamic Content of LHRH and TRH

Serum levels of pituitary hormones and hypothalamic content of LHRH and TRH were measured as described in "Materials and Methods" on the days of proestrus and estrus at 10 a.m. and 5 p.m. The results are summarized below for each hormone.

LH Results (Chart 1). In SD rats, there was a significant difference (p < 0.05) in serum LH between oil-treated and DMBA-treated animals on the afternoon of proestrus, on both the 5th and the 11th estrous cycles after treatment. Serum values of LH in DMBA-treated animals were lower than those in controls. In addition, the ratios of afternoon versus morning values on the 5th and the 11th estrous cycles were 14 and 15, respectively, for oil-treated animals but only 5 and 7 for DMBA-treated animals.

Regardless of the strain or treatment of animals or of the time after DMBA or oil treatment, the pattern of serum LH was the same, with a peak observed in the afternoon of proestrus; however, with DMBA treatment, this peak was reduced in SD animals.

FSH Results (Chart 2). As with LH, a significant decrease (p < 0.05) was noted in SD rats on the afternoon of proestrus after DMBA treatment. Again, this decrease was observed on both the 5th and the 11th estrous cycles after treatment. Furthermore, in SD animals treated with DMBA, there was no surge of FSH in the afternoon of proestrus, whereas a significant increase in serum FSH was found in oil-treated SD animals or in W rats of either treatment group.

PRL Results (Chart 3). As with LH and FSH, a significant

Table 1
Effect of DMBA or oil treatment on the regularity of estrous cycles in SD and W rats

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Short term</th>
<th>Long term</th>
<th>Incidence of mammary tumors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>DMBA</td>
<td>83</td>
<td>70</td>
<td>100*</td>
</tr>
<tr>
<td>SD</td>
<td>Oil</td>
<td>81</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>W</td>
<td>DMBA</td>
<td>19</td>
<td>9</td>
<td>10*d</td>
</tr>
<tr>
<td>W</td>
<td>Oil</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Up to the 3rd estrous cycle after treatment.
* Between the 6th and the 9th estrous cycle after treatment.
* All tumors appeared between the 13th and the 16th estrous cycle following treatment.
* Tumors appeared between the 13th and 75th estrous cycle following treatment.

Chart 1. Effect of DMBA on serum LH levels. Animals were treated with oil (open columns) or DMBA (stippled columns) and sacrificed on the days of proestrus and estrus of the 5th (A, B) and 11th (C, D) cycles. Serum levels of LH are shown for SD rats (A, C) and W rats (B, D). Each column represents the mean values of 5 animals; bars, S.E.; *, p < 0.05.
difference (p < 0.05) was also noted in SD rats between PRL serum levels of DMBA-treated and oil-treated animals. Contrary to gonadotropins, higher PRL values were observed in DMBA-treated animals than in controls. No significant difference between treatment groups was found at any time of proestrus or estrus in W rats. However, as we have previously reported (11), W rats exhibited a transient surge of PRL on the afternoon of estrus, which we did not observe in SD rats in this study. Inasmuch as this surge in W rats is very short lived, its significance is not readily demonstrated in these limited-time-interval studies.

GH Results (Chart 4). No significant difference was found for any time examined (i.e., proestrous and estrous stages, morning or afternoon) after DMBA treatment. Nevertheless, the amplitude of the fluctuation as well as the mean value of serum GH levels were lower in W than in SD rats. In addition, in SD rats morning values were always significantly higher than afternoon values, regardless of the stage of the cycle investigated or the treatment of animals; these differences were not always significant in W rats.

TSH Results (Chart 5). Because of technical reasons, TSH serum levels were only assayed on the 5th cycle after DMBA treatment. It can be seen that no significant difference was found between DMBA-treated and control animals at any time in the 2 days of the estrous cycle investigated either in SD or W rats. Furthermore, contrary to the results with GH, no marked differences were found between strains of animal with regard to mean TSH levels and the circadian rhythm.

LHRH and TRH Results. There was no difference in LHRH or TRH hypothalamic content between DMBA-treated and control animals of either strain (data not shown). Nevertheless, as we have reported previously for male rats (13), a well-marked rhythm was observed for both LHRH and TRH between morning and afternoon values (especially on the proestrous day), and this rhythm was not affected by DMBA treatment. Thus, the hypothalamic contents of LHRH and TRH were 1.5 ± 0.1 (S.E.) and 11.9 ± 0.4 ng/hypothalamus, respectively, for the morning and 2.1 ± 0.1 and 13.2 ± 0.5 ng/hypothalamus, respectively, for the afternoon.

Effect of DMBA on Serum Titers of Pituitary Hormones and Hypothalamic LHRH and TRH Content during the Afternoon of Proestrus

LH Results. Chart 6 shows profiles of serum LH levels between 1 and 7 p.m. on the day of proestrus during the 5th and 11th cycles after treatment. It can be seen that preovulatory surges occur during the same time interval in all cases (unfortunately, the end of the preovulatory surge was not...
DMBA Alters Preovulatory LH, FSH, and PRL Release in SD Rats

<0.025) using the Fisher-Snedecor F test in SD rats treated with DMBA than in those treated with oil. Furthermore, values of control groups of both strains did not differ significantly.

FSH Results. Chart 7 shows that there was no alteration in the timing of the FSH surge as a result of DMBA treatment. As with serum LH, the levels of serum FSH were significantly lower at 6 and 7 p.m. during the 5th estrous cycle and at 6 p.m. during the 11th estrous cycle. In addition, as with serum LH, the sum of the same serum FSH values at all times of the 5th and 11th estrous cycles was significantly lower (60% decrease, p < 0.025) in DMBA-treated animals than in controls. Furthermore, in spite of a tendency towards reduction, there was no significant difference between oil-treated animals of both strains.

observed in W rats). Serum values of LH were significantly lower (p < 0.05) at 6 and 7 p.m. during the preovulatory surge on the 5th cycle and at 6 p.m. during the 11th preovulatory surge after DMBA treatment in SD rats. In contrast, no significant difference was found at any time after treatment in W rats. The sum of the means of serum LH values for all times of both preovulatory surges was significantly lower (45% decrease, p

NOVEMBER 1979 4703

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alterations in hypothalamic or extrahypothalamic areas impli-
cation while the carcinogen acts solely as a mammary gland
feedback mechanism, put new demands on the neuroendo-
crine axis. Thus, developing tumors might alter neuroendocrine
duction by DMBA in SD rats, i.e., diminished LH and FSH
preovulatory surges and an increased PRL surge on the after-

PRL Results. Chart 8 shows that there was no alteration in
timing of the PRL surge; the surge initiated and lasted for the
same time in all cases. Contrary to serum gonadotropins, serum
PRL values were significantly higher at different times (4 p.m.
on the 5th estrous cycle and 4 and 5 p.m. on the 11th estrous
cycle) in DMBA-treated as compared to oil-treated SD rats.
The sum of the mean PRL values at all times of the afternoon
of the 5th and 11th estrous cycles was significantly higher
(90% increase, p < 0.05) in DMBA-treated animals than in
controls. No such difference was observed between DMBA-
treated and oil-treated animals in W rats; nevertheless, as for
serum FSH, there was a tendency towards a reduction in
control SD versus control W rats.

LHRH and TRH Results. There was no significant difference
in either LHRH or TRH hypothalamic content between DMBA-
treated and control animals in either strain at any time after

DISCUSSION

The present work demonstrates that the mammary carcino-
gen DMBA does provoke specific changes in the length of the
estrous cycle and in the preovulatory release of PRL and
gonadotropin in SD female rats. Female W rats are not suscep-
tible to DMBA treatment, and they do not show specific
changes in the length of the estrous cycle or in preovulatory
release of PRL and gonadotropin after treatment with the
carcinogen. The change in the length of the estrous cycle in
SD rats consisted of a specific lengthening of the cycle after
long-term treatment. In fact, both strains of animals, regardless
of treatment, exhibited a decreased cyclicity shortly after treat-
ment; however, this short-lasting, nonspecific deregulation was
much more pronounced in SD than in W rats (LHRH, 12.3 ± 0.9 ng/
hypothalamus in SD rats versus 7.3 ± 0.5 ng/hypothalamus in
W rats; TRH, 99.6 ± 2.0 in SD rats versus 88.4 ± 2.0 in W
rats).

In support of our contention, a direct effect of DMBA ster-
etaxically implanted in the preoptic area of the hypothalamus
was observed by Stern et al. (16). The authors reported a
change in vaginal cyclicity following this procedure. In addition,
when DMBA was administered at 50 days of age to neonatally
androgenized females, these sterile females were immediately
released from the constant estrous state (within 48 hr) and
began to exhibit sporadic cyclicity. Interestingly enough, Stern
et al. also observed that these animals which entered a dies-
trous stage of the estrous cycle developed mammary tumors of
the fibroadenoma, not adenocarcinoma, type. Thus, it would
appear that DMBA may have its effect directly on the hypo-
thalamus and that this effect may exert subsequent influences
at the mammary level.

With regard to the specificity of the deregulation, the possi-
bility that DMBA-treated rats may have altered timing of LH,
FSH, and PRL surges can be excluded, since the timing of
these preovulatory surges was the same in all animals. On the
other hand, changes in the length of the estrous cycle in
DMBA-treated SD rats further substantiate the hormonal im-
balance. The specific and transient hormonal changes were
not associated with consistent changes in hypothalamic LHRH
and TRH content. Nevertheless, without determinations of se-
rum LHRH and TRH, these negative results do not allow us to
conclude that the release of LHRH and TRH was not, respec-
tively, diminished and enhanced. Thus, reduced release of
LHRH and increased release of TRH would produce the ob-
served hormonal imbalance. One explanation for reduced go-
adotropin surges and an enhanced PRL surge would be to
postulate reduced sensitivity of the gonadotrophs to LHRH as
well as increased sensitivity of the mammotroph cells to TRH
or to other PRL secretagogues. This is rather unlikely, at least
for gonadotrophs, since LH and FSH were released to the
same extent with unmodified kinetics on the early afternoon
of proestrus after an i.v. injection of LHRH* in DMBA-treated
and untreated SD rats (the experiment was done on the 5th estrous
cycle after DMBA treatment). However, our data fit with recent
published data which showed that injection of LHRH agonists
does inhibit the growth of spontaneous (5) or DMBA-induced
mammary tumors (5, 9, 15). In addition, it has been reported
that injection of TRH in thyroidectomized animals produces a
significant increase in size and number of carcinogen-induced
mammary cancers via stimulation of PRL release from the
anterior pituitary (2). In view of the very short half-lives of these
2 hypothalamic hormones, these latter results clearly show that
transient pulses of exogenous LHRH and TRH can reverse or
accelerate the process of mammary tumorigenesis.

In agreement with these results, a stimulatory effect of DMBA
and other mammary carcinogens, 3-methylcholanthrene or 3-
methylcholanthrene has been described for PRL release in SD
rats (4, 14). Furthermore, serum PRL levels at diestrus in the
morning 20 days after DMBA administration have been ob-
served to be higher for SD rats than for Long-Evans rats, which
have a low incidence of mammary tumor formation (1, 7). Strain
differences such as these and those contained in this report
may provide a clue to the role of the neuroendocrine axis in
tumorigenesis. Thus, in addition to differential effects of DMBA,
we have observed surges in PRL on the afternoon of estrus in
W but not SD rats and higher content of LHRH and TRH in the
hypothalamus of SD rats when compared to W rats. Differences

* A. El Abed and B. Kerdelhue, manuscript in preparation.
such as these may relate to the differential sensitivity to DMBA observed for different genetic strains of animals. These differences may also play a role in the genesis of mammary tumors.

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