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 hormone and thyroliberin 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 a 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 hypothalamic-pituitary 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 mammotropic carcinogen, DMBA. It was further demonstrated by the same authors that DMBA must be adminis-
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, GH, 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

![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.](image_url)
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

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 mean 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.

Chart 5. Effect of DMBA on serum TSH levels. Animals were treated with oil (open columns) or DMBA (stippled columns) and sacrificed on the days of proestrus and estrus of the 5th cycles. Serum levels of TSH are shown for SD rats (A) and W rats (B). Each column represents the mean values of 5 animals; bars, S.E.

Chart 6. Effect of DMBA on the pattern of serum LH. Animals were treated with oil (○) or DMBA (●) and sacrificed at the times indicated on the afternoon of proestrus of the 5th (A, B) and 11th (C, D) cycles. Patterns of serum LH are shown for SD rats (A, C) and W rats (B, D). Each point represents the mean value of 5 animals; bars, S.E.; *, \( p < 0.05 \).

Chart 7. Effect of DMBA on the pattern of serum FSH. Animals were treated with oil (○) or DMBA (●) and sacrificed at the times indicated on the afternoon of proestrus of the 5th (A, B) and 11th (C, D) cycles. Patterns of serum FSH are shown for SD rats (A, C) and W rats (B, D). Each point represents the mean value of 5 animals; bars, S.E.; *, \( p < 0.05 \);

Chart 8. Effect of DMBA on the pattern of serum PRL. Animals were treated with oil (○) or DMBA (●) and sacrificed at the times indicated on the afternoon of proestrus of the 5th (A, B) and 11th (C, D) cycles. Patterns of serum PRL are shown for SD rats (A, C) and W rats (B, D). Each point represents the mean value of 5 animals; bars, S.E.; *, \( p < 0.05 \); **, \( p < 0.01 \); ***, \( p < 0.001 \).

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, \( p < 0.025 \)) 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.
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 treatment. Nevertheless, in controls, the sums of the means of preovulatory LHRH and TRH hypothalamic content were significantly higher 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).

DISCUSSION

The present work demonstrates that the mammary carcinogen 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 susceptible 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 treatment; however, this short-lasting, nonspecific deregulation was much more pronounced in SD than in W rats. This decreased cyclicity shortly after treatment may result from stress due to the gastric intubation method used for the administration of DMBA or oil. If so, it would appear that SD rats are more susceptible to this factor than are W rats. Nevertheless, this is not an argument against the role of DMBA in the deregulation of the estrous cycle seen between the 6th and 9th cycles posttreatment, since this deregulation existed only in DMBA-treated SD rats.

The specific hormonal imbalance associated with tumor induction by DMBA in SD rats, i.e., diminished LH and FSH preovulatory surges and an increased PRL surge on the afternoon of proestrus, suggests that an early and persistent deregulation was provoked by the carcinogen. The mechanism of this deregulation is difficult to define at this time. It is possible that developing mammary tumors, through some undefined feedback mechanism, put new demands on the neuroendocrine axis. Thus, developing tumors might alter neuroendocrine activity while the carcinogen acts solely as a mammary gland mutagen. However, in our opinion, DMBA may act primarily at the level of the central nervous system to provoke discrete alterations in hypothalamic or extrahypothalamic areas implicated in the cyclic response of the pituitary.

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 those animals which entered a diestrous 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 hypothalamus and that this effect may exert subsequent influences at the mammary level.

With regard to the specificity of the deregulation, the possibility 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 imbalance. The specific and transient hormonal changes were not associated with consistent changes in hypothalamic LHRH and TRH content. Nevertheless, without determinations of serum LHRH and TRH, these negative results do not allow us to conclude that the release of LHRH and TRH was not, respectively, diminished and enhanced. Thus, reduced release of LHRH and increased release of TRH would produce the observed hormonal imbalance. One explanation for reduced gonadotropin surges and an enhanced PRL surge would be to postulate reduced sensitivity of the gonadotrophs to LHRH as well as increased sensitivity of the mammatroph 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 recently 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 observed 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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REFERENCES


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