Eur J Cancer Clin Oncol, Vol. 19, No. 6, pp. 825–834, 1983.
Printed in Great Britain.

0277-5379/83$3.00+0.00
Pergamon Press Ltd.

Effects of Reserpine Administration on Rat Mammary Tumors and Uterine Disease Induced by *N*-Nitrosomethylurea*

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Abstract—*Reserpine 50 μg/100 g body weight administered s.c. to female rats on the day before, of and after each of three N-nitrosomethylurea (NMU) i.v. injections, influenced a number of experimental observations. The total doses of NMU given to the various treatment groups were 15, 12, 9, 6, 3 and 1.5 mg/100 g body weight. Tumor incidence ranged from approximately 100 to 0% in the control and reserpine-treated groups, depending on the dose of carcinogen given. Concurrent reserpine administration with the NMU decreased the number of tumors per rat; at 15 and 12 mg/100 g body weight NMU doses the values were only 58 and 55% of the respective controls. Reserpine treatment at these 2 NMU doses also increased the percentage of well-differentiated mammary tumors compared with corresponding controls. Tumor estrogen receptor levels in controls ranged from 71 to 78 fmol/mg protein and in the reserpine-treated groups from 96 to 105 fmol/mg protein; significant elevations were present in the rats given reserpine and the 2 highest doses of NMU. Tumor progesterone and prolactin receptor levels were similar in control and reserpine-treated groups. The estrous cycle of the controls was frequently arrested at estrus and 21/57 (37%) had a fluid-distended uterus and thickened uterine walls; only 2/52 (4%) of reserpine-treated rats had hydrometria and none exhibited cycle arrest at estrus. The effect of reserpine on mammary tumors was also studied when given after initial exposure to the carcinogen. NMU was administered to 65 rats in 3 doses of 5 mg/100 g body weight by i.v. injections 4 weeks apart. Equal numbers of animals comprised 1 control and 2 reserpine treatment groups. Reserpine was given at a daily dose of 20 μg/100 g body weight commencing 67 days after the first NMU dose (chronic regimen) or by the same schedule preceded by 50 μg/100 g doses given on the day before, with and on the day after the second and third NMU injections (acute/chronic regimen). A fourth group of rats received chronic reserpine treatment but no NMU. All 3 NMU-exposed groups had a 100% tumor incidence; none developed in the reserpine-treated controls. The acute/chronic reserpine treatment increased the rate of tumor development but neither drug schedule altered the number of tumors per rat or the mean final total tumor areas. Reserpine administration was associated with greater degrees of anaplasia in mammary tumors induced by NMU ($P < 0.01$). The mean tumor estrogen receptor content was significantly higher in the acute/chronic reserpine-treated group ($P < 0.0002$) and the chronic reserpine-treated animals ($P < 0.008$) compared with controls. Tumor prolactin receptor content was unaffected by the drug. Peroxidase activity was higher in mammary tumors and lower in uteri from reserpine-treated rats compared with tumor-bearing controls. Also, there was a strong correlation ($r = 0.82$) between the mammary tumor estrogen receptor and tumor peroxidase activity for rats treated with the acute/chronic reserpine regimen.*

Accepted 1 December 1982.
*Supported in part by USPHS Grant CA 14520 awarded to the Wisconsin Clinical Cancer Center by the National Cancer Institute, and by an award from the Elsa U. Pardee Foundation.
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INTRODUCTION

AN ASSOCIATION between the rauwolfia alkaloid reserpine and human breast cancer risk was first described by the Boston Collaborative Drug Surveillance Program [1]. Some further epidemiological investigations supported this finding [2, 3] while others did not [4–6], and more recent discussion suggests that there are additional issues to consider in evaluating these reports [7]. Armstrong *et al.* [8] interpreted their data from a breast cancer case-control study of hypertensive women as being consistent with rauwolfia derivatives exerting a promotional or stimulatory effect rather than initiating neoplastic transformation of mammary cells. However, a prospective study reviewed after a 5-yr observation period failed to show an increased diagnosis of breast cancer in patients receiving reserpine for the treatment of mild hypertension [9].

Serum prolactin levels are elevated in reserpine-treated rats [10] and women [11], but while prolactin stimulates the growth of chemically induced rat mammary tumors [12, 13], a role for this hormone in human breast cancer is much less certain [14]. Experimental evidence from animal models has demonstrated both enhancing and protective effects of reserpine in mammary carcinogenesis with dimethylbenz(a)anthracene (DMBA), depending on whether the drug is given before or after the carcinogen [15].

Rat mammary carcinomas induced by *N*-nitrosomethylurea (NMU) have gained favor recently as a model for human breast cancer. Tumorigenesis is inhibited by suppression of circulating prolactin levels [13] and, once established, the majority of these carcinomas require both prolactin and estrogens for their continued growth [16, 17]. The dosage schedule of NMU administration determines the histological characteristics of the tumors such that the higher the total dose given the greater the degree of anaplasia [18, 19].

The purpose of the present investigation was to study the effect of reserpine-induced hyperprolactinemia on the biological behavior of NMU-induced rat mammary carcinomas. In the first experiment reserpine was administered concurrently with 6 dosage levels of NMU. The second experiment was designed to observe the effect of reserpine when given at a time remote from the initial exposure to NMU; in one part of this study continuous daily administration of reserpine was commenced 4 weeks after the last of 3 doses of NMU and in the other single doses were given on the day before, with and after the second and third NMU injections and then chronic treatment started as before.

MATERIALS AND METHODS

Experiment one

Animals and tumor induction. The NMU was purchased from ICN Pharmaceuticals Inc., Plainview, NY and was used without further purification. Solutions at concentrations of 1, 2, 4, 6, 8 and 10 mg/ml were prepared just prior to use by dissolving the carcinogen in distilled water acidified with 3% acetic acid. Female Sprague-Dawley rats (King Laboratories, Oregon, WI) were purchased when 45 days old and allocated randomly to 1 of 12 treatment groups. There were initially 25 rats in each group. Six of the treatment groups received reserpine (Sigma Chemical Co., St. Louis, MO) 50 μg/100 g body weight in a 0.1 ml saline suspension by s.c. injection on the mornings before, of and after each of 3 NMU injections. The saline vehicle was given in place of the reserpine suspension to the other 6 groups, which also received the NMU. One of the following 6 doses of NMU was given i.v. to 1 control and 1 reserpine-treated group 3 times, 4 weeks apart, beginning when the animals were 50 days old (day 0): 0.5, 1.0, 2.0, 3.0, 4.0 or 5.0 mg/100 g body weight. Immediately before NMU administration the rats were anesthetized with ketamine and blood samples taken from the external jugular vein for prolactin assays. Unpublished studies from our laboratories have shown that ketamine, unlike many anesthetics, does not elevate the serum prolactin concentration in female rats. The rats were palpated every few days after the final NMU injection until measurable mammary tumors had developed. These were then measured weekly with a caliper. When the largest tumor present had achieved a maximum diameter of 2 cm or after 23 weeks since the first NMU injection a vaginal smear was taken each day until diestrus or for a total of 5 days. On the day of diestrus or after establishing the presence of persistent estrus all of the mammary tumors were excised under ether anesthesia. A portion of each tumor was placed in Warf fixative [18] and the rest frozen in 10 mM Tris, 1 mM EDTA, 0.25 M sucrose buffer, pH 8.0, at -70°C until assayed for estrogen receptor (ER), progesterone receptor (PgR) and prolactin receptor (PrLR) content. The animal was then killed and the uterus examined.

Histology, hormone receptor and serum prolactin assays. Tumor tissues were cut into 5-μm sections and stained with hematoxylin and eosin. On histological examination fibroadenocarcinomas and cystic papillary adenocarcinomas were classified as well-differentiated tumors, and mixed cystic papillary–medullary and medullary carcinomas as poorly differentiated tumors [18].

ER and PgR analyses of mammary tumors were performed as previously described [19]. Plasma membranes for PrLR determinations were isolated according to the method of Shiu and Friesen [20]. The 14,400-*g* pellets were resuspended in 0.025 M Tris–HCl, pH 7.6, with 0.1% bovine serum albumin and 0.01 M $MgCl_2$ to give a concentration of 2 mg/ml and frozen at −70°C. Single-dose receptor assays were performed in duplicate at 25°C as described by Holdaway and Friesen [21] except that a 24-hr incubation period was used, because in unpublished experiments we found a 23% greater binding with a 24-hr rather than the 6-hr incubation used by these authors. Ovine prolactin (NIH-510), a gift from the National Pituitary Agency, National Institutes of Health, was labeled with ^{125}I (New England Nuclear) by a lactoperoxidase method [20]. Approximately 80,000 counts/min of [^{125}I]-prolactin, sp. act. 75–100 μCi/μg, was added, in the presence or absence of 300 ng of unlabeled prolactin, to 200 μg of plasma membrane protein; the total volume was 0.5 ml. Prolactin binding was expressed as counts/min/mg cell membrane protein.

Serum prolactin was determined by double-antibody radioimmunoassay with materials provided by Dr. A. F. Parlow, National Hormone and Pituitary Program, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases; the reference preparation was NIADDK-rat prolactin RP-2 (biological potency approximately 30 IU/mg). Goat-to-rabbit antibody was purchased from Calbiochem-Behring Corporation, La Jolla, CA.

Experiment two

Forty-five-day-old Sprague–Dawley female rats were randomly assigned to 4 treatment groups. The NMU was prepared as before to give a concentration of 10 mg/ml. When the animals were 50 days old (day 0) the first of 3 NMU injections was given i.v. at a dose of 5 mg/100 g body weight to 3 treatment groups. The NMU was administered at intervals 4 weeks apart. On both the second and third NMU injections one group of 15 rats was treated with reserpine 50 μg/100 g body weight suspended in 0.1 ml physiological saline and injected s.c. on the morning before, with and after the NMU. On day 67 after the first NMU injection these animals started chronic reserpine treatment, at a daily dose of 20 μg/100 g body weight given 6 days a week for the duration of the study. A second NMU-exposed group of 15 animals received chronic reserpine treatment only, while a third received no reserpine and served as the NMU-treated control. A fourth group, comprised of 20 rats which did not receive NMU, was administered reserpine according to the chronic regimen only and served as reserpine-treated controls.

The rats were palpated every few days after the final NMU injection until measurable tumors had developed; these were then assessed weekly. When the largest tumor had a diameter of approximately 2.5 cm a vaginal smear was taken, the rat anesthetized with ether and the tumors excised. Animals failing to achieve this criterion were killed on day 154 of the study. A portion of each tumor was taken for histological examination and the remainder stored at −70°C until assayed for ER and peroxidase activity. When all tumors had been excised the animal was killed and a necropsy performed. The uterus was removed and frozen at −70°C in Tris buffer, pH 8.0, for peroxidase assay. Uterine and tumor peroxidase levels were determined by the method of Lyttle and De Sombre [22]. A unit of peroxidase activity was defined as the amount of enzyme required to produce an increase of 1 absorbance unit/min and results were expressed in units of absorbance/mg sonicate protein.

RESULTS

Experiment one

Mammary tumor incidence and multiplicity declined with decreasing doses of NMU in both the control and reserpine-treated groups, with the two lowest doses failing to induce tumor formation (Table 1). The concurrent administration of reserpine and NMU did not alter tumor incidence compared with NMU alone but did decrease the number of tumors per rat. This effect was greatest in the groups given the largest total doses of NMU, 15 and 12 mg/100 g body weight, where tumor multiplicity was reduced by reserpine treatment to 58 and 55% of the respective control values ($P<0.01$ and $P<0.05$). The greatest difference in histology was also seen at the two highest NMU doses, reserpine administration resulting in 49.1 and 44.8% of the tumors being well-differentiated while 94.4 and 87.3% ($P<0.001$ and $P<0.01$) of the corresponding control tumors were poorly differentiated (Table 2). But in addition, reserpine treatment at all doses of NMU caused approximately half of the tumors to be well-differentiated and half poorly differentiated, whereas in the controls the degree of anaplasia varied with the dose.

Overall, serum prolactin levels during tumor induction were higher at proestrus–estrus than at metestrus–diestrus. The mean serum prolactin concentration (±2 S.E.) for controls at proestrus (37.0 ± 23.9 ng/ml) was lower than that for proestrus reserpine-treated animals (73.0 ± 35.1 ng/ml). Control prolactin levels (33.0 ± 25.4

ng/ml) were also lower at estrus than were those of reserpine-treated rats (40.0 ± 44.9 ng/ml) but there were no differences at metestrus (2.1 ± 1.1 ng/ml and 2.5 ± 1.9 ng/ml) or diestrus (2.1 ± 1.3 and 3.1 ± 2.4 ng/ml). As reflected in the standard errors, there was considerable variation in the serum prolactin results, due largely to unavoidable differences in the time of day at which the samples were obtained. Consequently the observed differences between groups were not statistically significant.

The results of the PrLR assays of mammary tumors from control and reserpine-treated animals are given in Table 3. There was a large variance in the specific binding of [^{125}I]-prolactin, and the differences for corresponding NMU doses were not significant.

Table 4 compares the ER and PgR levels and

Table 1. Influence of concurrent reserpine treatment and varying dose of NMU on tumor incidence and number per rat*

Total dose of NMU†	Tumor incidence (%)‡		Average No. of tumors per rat	
	Controls	Reserpine-treated	Controls	Reserpine-treated
15	100 (23)	95 (21)	4.18	2.43§
12	72 (25)	67 (24)	2.35	1.29
9	54 (24)	56 (25)	0.96	0.76
6	33 (24)	40 (25)	0.38	0.52
3	0 (25)	0 (25)	0	0
1.5	0 (25)	0 (25)	0	0

*Reserpine 50 g/100 g body weight was given on the day before, of and after each of 3 NMU i.v. injections.

†Total dose is the sum of 3 individual doses (mg/100 g body weight) given 1 month apart.

‡The number of rats in each group is given in parentheses.

§Significantly smaller than corresponding control group, $P < 0.01$.

Table 2. NMU-induced mammary tumor histology: effect of reserpine when administered in combination with NMU*

Total dose of NMU		No. of tumors examined	Well-differentiated tumors (%)	Poorly differentiated tumors (%)
15	Control	89	5.6	94.4
	Reserpine	51	49.1	51.1
12	Control	40	12.5	87.5
	Reserpine	29	44.8	55.2
9	Control	24	41.7	58.3
	Reserpine	17	64.7	35.3
6	Control	9	66.8	33.2
	Reserpine	12	50.0	50.0

The differences in frequency of well-differentiated and poorly differentiated tumors with and without reserpine treatment were statistically significant for the 15 mg/100 g ($P < 0.001$) and the 12 mg/100 g ($P < 0.01$) body weight doses of NMU.

*Well-differentiated tumors include fibroadenocarcinomas and cystic papillary adenocarcinomas. Poorly differentiated tumors all have medullary areas, and include mixed cystic papillary–medullary tumors as well as purely medullary carcinomas.

Table 3. Prolactin receptors (PrLR) in mammary tumors induced by NMU with and without concurrent reserpine treatment

Total dose of NMU	PrLR (counts/min/mg membrane protein) (mean ± S.E.M.)	
	Controls (No.)	Reserpine-treated (No.)
15	25734 ± 2735 (16)	35963 ± 8006 (17)
12	23814 ± 2966 (14)	24964 ± 4645 (15)
9	22370 ± 3856 (12)	26480 ± 3921 (13)
6	14170 ± 2700 (11)	33182 ± 8423 (10)

There were no significant differences between the control and reserpine-treated groups.

*Table 4. Estrogen receptor (ER) and progesterone receptor (PgR) levels and PgR/ER ratio in mammary tumors induced by NMU with and without concurrent reserpine treatment (mean ± S.E.M.)**

Total dose of NMU	ER (fmol/mg protein)		PgR (fmol/mg protein)		PgR/ER	
	Controls	Reserpine-treated	Controls	Reserpine-treated	Controls	Reserpine-treated
15	78 ± 4 (24)	99 ± 8 (38)†	222 ± 16	264 ± 3	3.6 ± 0.4	3.6 ± 0.6
12	77 ± 9 (19)	103 ± 8 (26)‡	342 ± 37	257 ± 28	6.0 ± 0.7	2.8 ± 0.3§
9	76 ± 11 (10)	96 ± 8 (13)	308 ± 59	231 ± 46	4.9 ± 0.6	2.4 ± 0.4‖
6	70 ± 15 (7)	105 ± 14 (10)	283 ± 116	364 ± 66	4.5 ± 2.2	3.2 ± 0.3

*The numbers in parentheses refer to tumors assayed for both ER and PgR.
†Significantly higher than the corresponding control ER values, $P < 0.04$.
‡Significantly higher than the corresponding control ER values, $P < 0.02$.
§Significantly lower than the corresponding control ratio, $P = 0.02$.
‖Significantly lower than the corresponding control ratio, $P = 0.05$.

the PgR/ER ratios for mammary tumors in the control and reserpine-treated groups. The ER and PgR levels did not vary with the dose of NMU. Mammary tumor ER in reserpine-treated rats ranged from 96 to 105 fmol/mg supernatant protein, while the control values ranged from 71 to 78 fmol/mg protein. The ER levels were significantly higher in reserpine-treated animals than the corresponding controls at the 2 highest doses of NMU ($P < 0.04$ and $P < 0.02$ respectively). The mean PgR/ER ratios in reserpine-treated rats exposed to 12 or 9 mg NMU/100 g body weight were significantly lower than in the corresponding controls (Fig. 1; $P = 0.02$, $P = 0.05$ respectively) because of increases in the ER levels.

Vaginal smears showing persistent estrus over a 5-day period occurred in 21 of 57 (37%) control rats but in only 2 of 52 (4%) reserpine-treated rats (Table 5). In most cases of persistent estrus the uterus was distended with fluid which was sometimes blood stained, and there was pronounced thickening of the uterine walls. These abnormalities were particularly common in the controls given 12 mg/100 g body weight total dose of NMU, being present in 9 of 16 (56%) animals whereas they were not seen in any of the corresponding reserpine-treated group ($P < 0.05$).

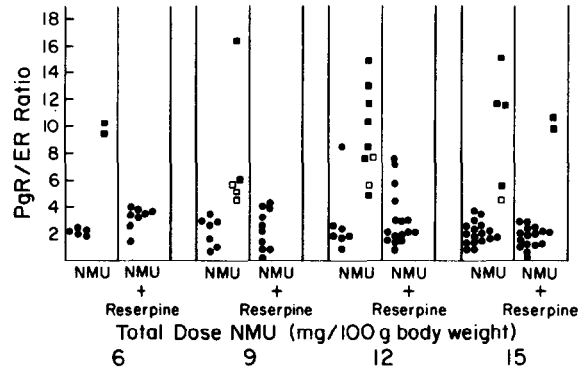

Fig. 1. PgR/ER ratios in reserpine-treated and control rats exposed to 1 of 4 different doses of NMU. • Normal estrous cycles; □ prolonged estrus and normal-appearing uteri; ■ prolonged estrus and fluid-distended uteri.

Experiment two

All of the NMU-treated groups had a 100% tumor incidence by day 84, whereas none had developed in the reserpine only-treated controls by the last day of the experiment (Table 6). During the first half of the study the rate of tumor appearance was most rapid in the acute/chronic reserpine-treated animals, so that tumor incidence was higher for this group by day 67 ($P < 0.01$). This trend persisted until all of the rats in the

*Table 5. Uterine abnormalities in control and reserpine-treated, tumor-bearing rats**

Total NMU dose mg/100 g body weight	Group	No. of rats examined	No. with estrous cycle and uterine abnormalities	No. with estrous cycle abnormalities only
15	Controls	22	4	1
	Reserpine-treated	18	2	0
12	Controls	16	7	2
	Reserpine-treated	16	0	0
9	Controls	12	2	3
	Reserpine-treated	10	0	0
6	Controls	7	2	0
	Reserpine-treated	8	0	0

*Rats without tumors had no estrous cycle or uterine abnormalities.

Table 6. Tumor development with and without reserpine treatment after exposure to N-nitrosomethylurea (NMU)

Treatment group	Tumor-bearing rats/ total No. of rats	Time to first tumor (weeks)	Termination time (weeks)‡	Final No. of tumors/rat	Mean total tumor area/rat (cm^2)
Reserpine only*	0/20	0	22.00 ± 0.00	0	0
NMU only	15/15	10.80 ± 0.4	14.87 ± 0.7	6.2 ± 0.6	14.02 ± 1.5
Chronic reserpine*–NMU	14/14	10.79 ± 0.4	15.29 ± 0.9	6.3 ± 0.9	14.70 ± 1.6
Acute/chronic reserpine†–NMU	14/14	9.86 ± 0.2	13.07 ± 0.7	6.5 ± 0.7	12.82 ± 1.3

The values are mean ± 2 S.E.
*20 μg/100 g body weight/day s.c. commencing on day 67 after the first NMU injection.
†50 μg/100 g body weight s.c. given on the day before, with and on the day after the second and third NMU injections, and the chronic schedule as detailed above.
‡When the largest tumor diameter was 2.5 cm or 22 weeks after first NMU injection: whichever came first.

group had developed tumors, but it was no longer statistically significant by day 72. Reserpine did not influence the number of tumors borne by each rat nor the final mean total tumor area per rat (Table 6).

Histological examination showed that reserpine treatment was associated with greater degrees of anaplasia in the NMU-induced mammary carcinomas compared with the tumors from controls which received only NMU (Table 7, $P < 0.002$). Approximately 25% of the tumors from reserpine-treated rats consisted of unorganized masses of cells, with extreme pleomorphism and many mitotic figures; these were classified as medullary carcinomas.

The mammary tumor ER levels were significantly higher in both the acute/chronic ($P < 0.0002$) and chronic ($P < 0.008$) reserpine-treated groups compared with controls, but the drug had no effect on prolactin binding (Table 8). Figure 2 shows the individual ER results. While the range of values was greater for the chronic reserpine-treated group, the mean receptor concentration was higher in those receiving the acute/chronic treatment. The net result was that there was no difference between the two. There was no statistically demonstrable relationship

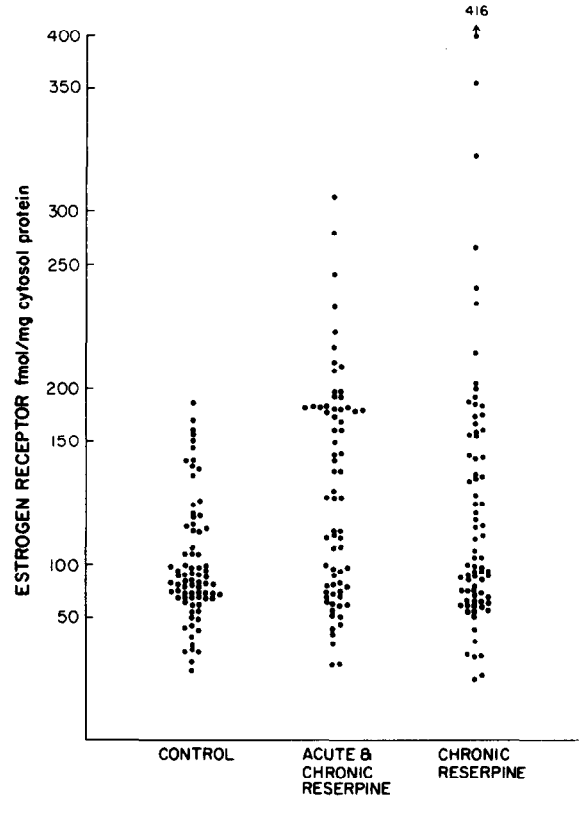

Fig. 2. Tumor estrogen receptor levels in untreated controls, chronic reserpine administration and an acute/chronic reserpine regimen (the dosage schedules are described in Table 6).

Table 7. N-nitrosomethylurea-induced mammary tumors with or without reserpine treatment, classified histologically according to the degree of anaplasia†*

Treatment group	Total No. of tumors in treatment group	Cystic papillary adenocarcinomas (%)	Cystic papillary/ medullary mixed (%)	Medullary (%)
NMU only controls	80	39 (48.8)	39 (48.8)	2 (2.4)
Chronic reserpine–NMU	72	24 (33.3)	32 (44.5)	16 (22.2)
Acute/chronic reserpine–NMU	56	8 (14.3)	32 (57.1)	16 (28.6)

*The cystic papillary adenocarcinomas were the most differentiated tumors, while the medullary carcinomas had the greatest degree of anaplasia.

†A 3×3 χ^2 test showed a significant difference in the degree of anaplasia between the NMU controls and the reserpine-treated groups ($P < 0.002$), with the greatest difference being for the acute/chronic reserpine-treated group.

Table 8. Mammary tumor hormone receptors and peroxidase activity (mean ± 2 S.E.) in uteri and tumors from NMU-exposed rats with or without reserpine treatment

Treatment group	Prolactin receptors (counts/min/mg protein)	Estrogen receptors (fmol/mg protein)	Peroxidase activity* Uterine	Peroxidase activity* Tumor	Ratio uterine/tumor
NMU only controls	19,471 ± 2060	87 ± 4	8.3 ± 1.6	0.6 ± 0.1	15.9 ± 2.8
Chronic reserpine–NMU	17,220 ± 2288	118 ± 11†	5.7 ± 2.7	1.5 ± 0.6	4.7 ± 1.4§
Acute/chronic reserpine–NMU	16,356 ± 2580	124 ± 8‡	6.8 ± 2.4	0.9 ± 0.3	14.5 ± 6.1

*Peroxidase activity is in units/mg sonicate protein; 1 unit of activity is the amount of enzyme required to produce an increase of 1 absorbance unit/min.

†Significantly higher than control group, $P < 0.008$.

‡Significantly higher than control group, $P < 0.0002$.

§Significantly lower than control group ratio, $P < 0.01$.

between tumor ER concentration and histological differentiation for the control or acute/chronic reserpine-treated groups, but the numbers in the various subgroups were small; in the chronic reserpine-treated group the cystic papillary adenocarcinomas had significantly higher ER values than the mixed cystic papillary–medullary or the medullary carcinomas ($P<0.05$).

Table 8 also shows the uterine and tumor peroxidase results. There was a trend for uterine peroxidase activity to be higher and tumor peroxidase lower in the control compared with the chronic reserpine-treated animals, such that the uterine/tumor activity ratio was significantly higher for the control group ($P<0.01$). At proestrus–estrus the uterine and tumor peroxidase activities in NMU-exposed controls showed a strong positive correlation ($r=0.77$), but this relationship was lost at metestrus–diestrus ($r=0.16$). Similarly, tumor ER and peroxidase activity in this group were positively correlated at proestrus–estrus ($r=0.65$) but to a lesser degree at metestrus–diestrus ($r=0.46$). While the relationship between tumor ER and peroxidase was lost in the controls when the stage of the estrous cycle was disregarded, a strong positive correlation was maintained in the acute/chronic reserpine-treated animals ($r=0.82$).

DISCUSSION

Welsch and Meites [15] studied the effect of pretreatment with reserpine on rat mammary carcinogenesis induced by dimethylbenz(a) anthracene (DMBA). When the drug was given for 30 days before DMBA administration it caused accelerated development of the immature mammary tissue and reduced the number of tumors which subsequently appeared in the individual rat. This protective effect of tissue maturation may have arisen because it exceeded the state of optimal cellular sensitivity for tumor induction [23]. Although the results from experiment 1 concerning the effect of concurrent reserpine administration on tumor multiplicity are similar to those reported by Welsch and Meites, it seems unlikely that a similar degree of mammary development could have been attained after a single dose of the drug one day before exposure to NMU.

In addition to decreasing tumor multiplicity, experiment 1 showed that bridging the time of carcinogen exposure by 1 day with reserpine treatment causes a shift towards the development of well-differentiated tumors, perhaps because the drug affects mammary cellular structure and function. Alternatively, the reserpine may stimulate hepatic microsomal enzyme activity and hence increase the NMU degradation rate so that there is a reduction in the effective dose of this direct-acting carcinogen. However, although we have shown previously that the smaller the dose of NMU the lower the incidence and the more differentiated the tumors [19], treatment with reserpine did not influence tumor incidence in the present study.

Whether the degree of differentiation in the mammary epithelium of 50-day-old rats or the hormonal milieu favors tumor induction is unclear [23], but it is likely that both are involved to some extent. Lindsey *et al.* [24] investigated the influence of the estrous cycle on mammary tumor induction by NMU and did find that animals exposed to the carcinogen during diestrus developed fewer tumors per rat, and with longer latent periods, than rats exposed at proestrus. Reserpine is known to prolong diestrus in the rat [25] and it is possible that in our study animals in the reserpine-treated group were largely in diestrus during the time of NMU exposure. This may explain why approximately equal proportions of tumors were well-differentiated and poorly differentiated at all four doses of NMU in the drug-treated rats while in the controls the degree of anaplasia varied directly with the dose of carcinogen.

Other differences between the controls and the rats treated acutely with reserpine may have been due to hormonal influences at the time of NMU administration. We reported recently that elevated mammary tumor PgR/ER ratios, constant estrus and endometrial hyperplasia with hydrometria occur frequently in rats exposed to this carcinogen [19]. These abnormalities are associated with polycystic disease of the ovaries [19] and a suppression of the serum progesterone levels which permits unopposed, excessive estrogenic stimulation of the endometrium [26]. The experiments were performed because of the incidental finding of uterine pathology on gross examination of control animals in the present study. Unfortunately neither the ovaries nor uteri were preserved for histological evaluation, but it appears likely that reserpine administration in some way modified the NMU-induced endocrinopathy, so preventing the development of endometrial hyperplasia. Further studies are indicated to determine whether reserpine exerts its protective effect at the level of the ovaries or the endometrium and to determine the contribution made by the known effects of this drug on circulating prolactin and gonadotropins.

When reserpine was given after initial exposure to NMU (experiment 2) it caused the rate of tumor appearance to be accelerated, with an increased

frequency of anaplastic carcinomas. Again, in the study by Welsch and Meites [15] chronic treatment with reserpine starting 75 days after DMBA exposure increased the average number of mammary tumors per rat. The presence of ER in human breast cancers is associated with well-differentiated tumors [27, 28], but we found previously that this was not the case in NMU-induced rat mammary carcinomas [18, 19]. Prolactin increases the ER level in rat mammary tumors, and Vignon and Rochefort [29] proposed that by so doing it sensitizes the neoplastic cells to the action of estrogen. In this study chronic reserpine administration produced an increase in ER concentration, presumably prolactin-related, but also resulted in more anaplastic-appearing tumors. However, in the chronic reserpine-treated group the cystic papillary adenocarcinomas had higher ER levels than the anaplastic carcinomas, suggesting that the prolactin-mediated effect of reserpine on ER was selective for the well-differentiated tumor type.

Not only were the ER levels elevated in the chronic reserpine-treated rats but this correlated with tumor peroxidase activity, indicating that there was, indeed, increased estrogenic stimulation of specific protein production. Mammary tumor peroxidase activity was higher and uterine peroxidase activity lower in reserpine-treated rats than in the controls, such that there was a significant difference in the uterine/tumor ratios. Uterine peroxidase activity is estrous cycle-dependent, being highest at proestrus–estrus and lowest at metestrus–diestrus [30]. It seems possible that tumor peroxidase is relatively insensitive to the rapid estrogen changes of the estrus cycle and better reflects prolonged stimulation, as is inferred by the high correlation of tumor ER and peroxidase in the chronic reserpine-treated animals.

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