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Effects of Ergocornine and Ergocryptine on Growth of 7,12-Dimethylbenzanthracene-induced Mammary Tumors in Rats[1]

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SUMMARY

The effects of ergocornine and ergocryptine on growth of 7,12-dimethylbenzanthracene-induced mammary tumors were investigated in female Sprague-Dawley rats. Sixty-eight rats with tumors were divided into 5 groups, and 3 groups were given s.c. injections every day for 4 weeks with ergocornine or ergocryptine; 1 intact and 1 ovariectomized group served as controls. The intact controls showed a significant increase in number and size of tumors throughout the treatment period, whereas ergocornine produced a significant reduction in number and size of tumors, paralleling the effects seen in the ovariectomized controls. Ergocryptine suppressed mammary tumor growth throughout the treatment period but produced no significant decrease in number and size of tumors. When ergocornine treatment was terminated at the end of 4 weeks, prompt renewal of mammary tumor growth was observed. Inhibition of mammary tumor growth in rats by these drugs is believed to be due to their demonstrated suppression of pituitary prolactin secretion.

INTRODUCTION

Ergocornine was reported to inhibit pseudopregnancy and early pregnancy (10), deciduoma formation (9), and lactation in rats (14). Our laboratory also observed in a preliminary experiment that Ec[4] inhibited growth of DMBA-induced mammary tumors in rats when injected daily for a 15-day period (7). Ec significantly decreased pituitary and serum prolactin levels and was shown to act both via the hypothalamus and directly on the pituitary (4, 7, 12). Inasmuch as prolactin is considered to be essential for mammary tumor growth in rats (1, 5, 6, 8, 11), it was of interest to determine further the effects of Ec and a related ergot drug, Ecy, on growth of DMBA-induced mammary tumors.

MATERIALS AND METHODS

All animals used in this study were virgin female Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, Mich.). They were housed in a temperature (75 ± 2°F)- and light (14 hr/day)-controlled room and given a diet of Wayne Lab Blox (Allied Mills, Inc., Chicago, Ill.) and water *ad libitum*. At 57 days of age, the rats were each given a single i.v. injection of 1 ml of a lipid emulsion containing 5 mg of DMBA[5] as described by Huggins (3). By 140 days of age, 68 rats had tumors that were 10 to 20 mm in diameter, and they were then divided into 5 groups and given injections s.c. daily for 28 days, as follows: Group 1, intact controls, 0.87% NaCl-ethanol, 16 rats; Group 2, 0.4 mg Ec/100 g of body weight in 0.87% NaCl-ethanol for the 1st 5 days and 0.2 mg Ec/100 g of body weight thereafter, 13 rats; Group 3, 0.2 mg Ec/100 g of body weight in 0.87% NaCl-ethanol, 15 rats; Group 4, 0.4 mg Ecy/100 g of body weight in 0.87% NaCl-ethanol, 15 rats; Group 5, ovariectomized controls, 0.87% NaCl-ethanol, 9 rats. In Group 2, the higher dose of Ec was replaced with the lower dose at the end of 5 days because the rats had rough hair coats and were less active than the control rats.

Ec and Ecy were prepared by dissolving the corresponding mesylate salts[6] in 100% ethanol and diluting with 0.87% NaCl solution so that the final concentrations were 1.0 mg Ec/ml or 2.0 mg Ecy/ml. Ethanol constituted approximately 15% of the volume of the final solutions. Control animals (intact and ovariectomized) were given injections of the same volumes of diluent (15% ethanol–0.87% NaCl solution).

Beginning on the 1st day of drug treatment and once a week thereafter, the number of tumors, mean tumor diameter, and body weight were recorded for each rat. With the use of a vernier caliper, only the largest diameter of each tumor was recorded. When palpable tumors regressed to such a small size that calipers could not be used accurately, an estimation of tumor size was made. Measurements were also recorded for a 3-week posttreatment period in the control and Ec-treated rats. Significance of differences between mean tumor diameters, mean number of tumors, and mean body weights were calculated by Student's *t* test.

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[4] The abbreviations used are: Ec, ergocornine; DMBA, 7,12-dimethylbenzanthracene; Ecy, ergocryptine.

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[5] DMBA emulsion was kindly made available by Dr. J. Hinman, The Upjohn Company, Kalamazoo, Mich.

[6] Ec mesylate and Ecy mesylate (CB-154) were provided through the courtesy of Dr. M. Taeschler and Professor E. Flückiger, Sandoz Ltd., Basel, Switzerland.

RESULTS

Ec and Ecy significantly suppressed mammary tumor growth throughout the 28-day period of treatment, and, in addition, Ec induced significant regression of number of mammary tumors. By the end of the 4-week treatment period, mean tumor diameter (Chart 1) in the intact control rats was 18.0 ± 1.2 mm in contrast to 6.8 ± 3.0 mm in the rats given 0.4 and 0.2 mg Ec/100 g of body weight, 7.0 ± 1.5 mm in the rats given 0.2 mg Ec/100 g of body weight, 16.6 ± 1.7 mm in the rats given 0.4 mg Ecy/100 g of body weight and 9.5 ± 4.4 mm in the ovariectomized controls. Tumor size was initially greater in the ovariectomized rats than in the other groups. The number of tumors (Chart 2) at the end of 4 weeks of treatment averaged 5.7 ± 1.1 in the intact controls, 2.6 ± 0.6 in the rats given 0.4 and 0.2 mg Ec/100 g of body weight, 1.5 ± 0.3 in the rats given 0.2 mg Ec/100 g of body weight, 3.5 ± 0.9 in the rats given 0.4 mg Ecy/100 g of body weight, and 1.2 ± 0.4 in the ovariectomized controls. The reduction in average number of tumors by Ecy was not statistically significant. The decrease in average size and number of mammary tumors elicited by ovariectomy appeared to parallel the effects of the Ec treatments and was more effective than treatment with Ecy.

Table 1 shows the effects of the different treatments on the state of the individual tumors at the end of 28 days. These tumors initially were all greater than 10 mm in diameter. In the intact controls, 17 tumors were still growing and only 2 tumors showed a reduction in size by the end of treatment. By contrast, in the 2 Ec-treated groups, most of the tumors disappeared or were in a state of regression, 2 tumors remained unchanged in size, and only 6 tumors showed continued growth. It is evident that Ecy was less effective than Ec in depressing tumor growth. Ovariectomy apparently was more effective than treatment with the ergot drugs, since all the tumors were either in a state of regression or had disappeared.

After treatment with the 2 drugs was terminated on the 28th day, the rats given Ecy were killed and tumor measurements on all other groups were continued for an

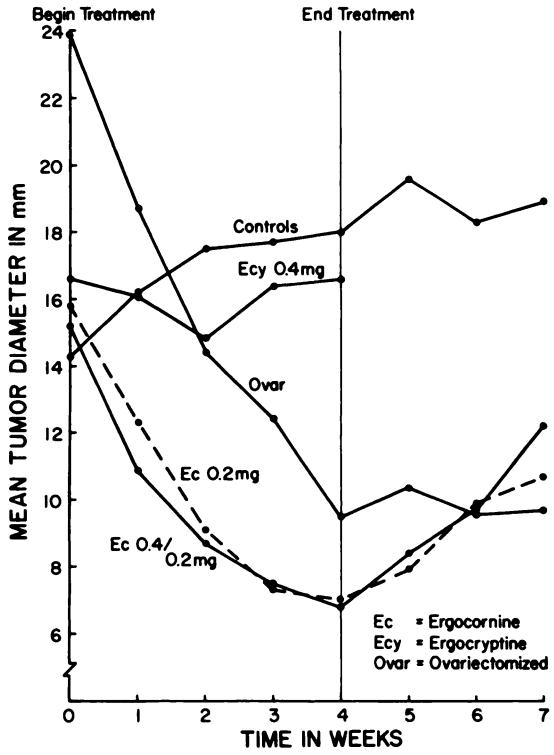

Chart 1. Mean mammary tumor diameter in female Sprague-Dawley rats given daily injections for 4 weeks of Ec or Ecy as compared with intact and ovariectomized controls. Note resumption of mammary tumor growth after Ec treatment was terminated.

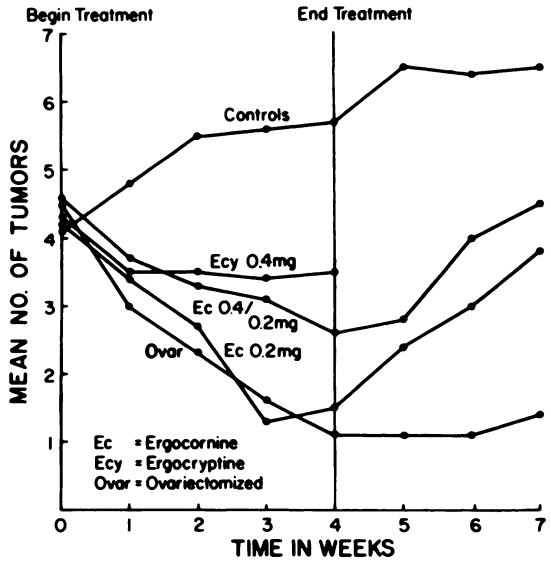

Chart 2. Mean number of mammary tumors in female Sprague-Dawley rats given daily injections for 4 weeks of Ec or Ecy. Note increase in number of tumors after treatment with Ec was terminated.

Table 1

Effects of Ec and Ecy on mammary tumor growth

These tumors initially were greater than 10 mm in diameter.

Treatment	No. of rats	No. of tumors in each category at end of treatment			
		Disappeared	Regressing	Unchanged	Progressing
Controls	16	0	2	0	17
Ec 0.4 and 0.2 mg	13	11	18	1	4
Ec 0.2 mg	15	10	6	1	2
Ecy 0.4 mg	15	0	10	6	8
Ovariectomized	9	14	8	0	0

additional period of 3 weeks. In the intact controls, mean tumor diameter and average number of tumors continued to show small increments, whereas the 2 Ec-treated groups showed marked increases in mean tumor diameter and average number of tumors. Mean tumor diameter in the 2 Ec-treated groups increased from 6.8 ± 3.0 and 7.0 ± 1.5 mm, respectively, to 12.2 ± 2.0 and 10.7 ± 2.1 mm, respectively. This growth is comparable to that of the intact controls during the 1st 3 weeks of the experiment. The ovariectomized controls showed no further reduction in mean tumor diameter or average number of tumors during the posttreatment period.

No significant differences in body weight were observed in any of the groups at the end of the 4-week treatment period. There were no apparent effects of Ec or Ecy on the health of these animals as compared to the control rats.

DISCUSSION

This study shows that daily injections of Ec or Ecy for 4 weeks suppressed growth of DMBA-induced mammary tumors in rats and that Ec also produced complete disappearance of some of these tumors. This confirms the preliminary report by Nagasawa and Meites (7) that daily injections of Ec for 15 days suppressed growth of DMBA-induced mammary tumors in Sprague-Dawley rats, although mammary tumor regression was not observed. At the doses used, Ec appeared to be more effective than Ecy in suppresssing mammary tumor growth. The dramatic increase in size and number of mammary tumors after Ec treatment was terminated indicates that Ec was responsible for the inhibitory effects on mammary tumor growth. At the doses used, neither Ec nor Ecy adversely affected body weight, in agreement with previous findings on the effects of Ec in rats (7, 12). Larger doses of these and other ergot drugs may be toxic to rats (2).

We recently observed that treatment with Ec or Ecy suppressed growth of spontaneous mammary tumors in old Sprague-Dawley female rats, and these showed resumption of growth when treatment with Ec or Ecy was terminated (K. Quadri and J. Meites, unpublished data). In addition, Yanai and Nagasawa (13) recently reported that Ec suppressed formation in C3H/He mice of hyperplastic alveolar nodules, believed to represent the preneoplastic state of mammary tumorigenesis in mice.

Inhibition of mammary tumor growth by the ergot drugs is believed to be produced by suppression of pituitary prolactin secretion. Evidence from our laboratory indicates that injections of Ec in rats depress serum prolactin levels throughout the estrous cycle and decrease pituitary prolactin content without altering the regularity of the estrous cycles (12). Ecy also decreases serum prolactin concentration in rats (E. E. Cassell and K. Quadri, unpublished data). Apparently, these effects are mediated via the hypothalamus and by a direct action on the anterior pituitary, since injections of Ec increase hypothalamic prolactin-inhibiting factor content (12) and directly inhibit prolactin synthesis and release by the pituitary under *in vivo* and *in vitro* conditions (4).

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