THE EFFECTS OF HORMONES AND DRUGS ON THE GROWTH OF CARCINOGEN-INDUCED MAMMARY TUMORS IN RATS

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ABSTRACT

THE EFFECTS OF HORMONES AND DRUGS ON THE GROWTH
OF CARCINOGEN-INDUCED MAMMARY TUMORS
IN RATS

by

James L. Clark

The effects of hormones and drugs on carcinogen-induced mammary tumors in rats were investigated in order to test further the current theory that prolactin plays a major role in promotion of mammary tumor growth. Some of the drugs used are known to alter catecholamine content of the hypothalamus and also to alter prolactin secretion. All agents employed in these studies influenced prolactin secretion and thereby caused inhibition or stimulation of mammary tumor growth.

1. Estradiol benzoate (EB) administered daily in doses of 20 μg for 20 days completely inhibited growth of mammary tumors induced by the carcinogen 7,12-dimethylbenzanthracene (DMBA), whereas mammary tumors in control rats increased by about 50% in diameter. When 1 mg of exogenous prolactin was administered simultaneously with 20 μg of EB, the inhibitory effect of EB was overcome completely and mammary tumor diameters increased about 50%. The inhibitory action of EB upon mammary tumor growth appears to be exerted via inhibition of the peripheral action of prolactin on the mammary gland. Administration of exogenous prolactin (1 mg) overcomes this inhibition by "flooding" the mammary tissue with prolactin.

2. Ovine prolactin (1 mg) injected daily for 3 weeks significantly increased growth of mammary tumors. Androgen treatment for 3 weeks (5 mg testosterone propionate for 1 week, followed by 5 mg of 11β-hydroxy-17-methyltestosterone for 2 weeks) had no significant effect mammary tumor growth. L-dopa (10 mg) for 3 weeks did not affect mammary tumor growth. Both Iproniazid (15 mg daily, reduced to 5 mg) and Pargyline (10 mg daily, reduced to 5 mg) prevented an increase in the growth of DMBA-induced mammary tumors. The dose of L-dopa may not have been sufficient to reduce prolactin secretion long enough to influence mammary tumor growth. The androgens are believed to have been given in insufficient doses to inhibit growth of mammary tumors.

3. Daily injections of haloperidol (150 μg) for 3 weeks enhanced growth of DMBA-induced mammary tumors. Lysergic acid diethylamide (LSD) (6 μg) and ergonovine (3.6 mg) tended to inhibit mammary tumor growth while Pargyline (6 mg) completely inhibited mammary tumor growth. Haloperidol has been reported to decrease hypothalamic catecholamines, thereby increasing prolactin release from the anterior pituitary gland. Pargyline interferes with prolactin release by increasing hypothalamic catecholamine and prolactin inhibiting factor (PIF) contents. LSD and ergonovine are believed to act directly on the anterior pituitary gland to inhibit prolactin secretion. This investigation supports the theory that prolactin is the major influence in promotion of mammary tumor growth in rats.

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By

James L. Clark

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Dedicated to

Tina, Tom, and Tip

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INTRODUCTION

Less than 20 years ago it was demonstrated that the hypothalamus exerted a regulatory influence on anterior pituitary function (Harris, 1955). Today it is known that the hypothalamus produces hypophysiotropic hormones which control the release and perhaps synthesis of the six hormones of the anterior lobe of the pituitary gland (Ganong, 1966; Meites, 1970; Burgus and Guillemin, 1970). Thus, cortiocotropin releasing factor (CRF) from the hypothalamus stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary (Saffran and Schally, 1955). In a similar manner, luteinizing hormone releasing factor (LRF) stimulates luteinizing hormone (LH) release (McCann et al., 1960), thyrotropin releasing factor (TRF) stimulates thyroid stimulating hormone (TSH) release (Guillemin et al., 1962), prolactin inhibiting factor (PIF) inhibits prolactin release (Meites et al., 1961; Pasteels, 1962), growth hormone releasing factor (GRF) stimulates growth hormone (GH) release (Deuben and Meites, 1964), and follicle-stimulating hormone releasing factor (FRF) stimulates follicle-stimulating hormone (FSH) release (Igarashi and McCann, 1964; Mittler and Meites, 1964).

These hypophysiotropic agents travel via axons from various nuclei in the hypothalamus to the storage terminals in the median eminence (Kobayashi and Matsui, 1969; Sulman, 1970). It is here that the secretory granules containing these factors enter the portal circulation destined for the cells of the anterior pituitary gland. The

special nature of the blood vessel walls in this region enables the granules to enter the circulation easily (Clementi and Ceccarelli, 1970). This is the "final common pathway" to the adenohypophysis.

Many drugs and hormones cause an increase or decrease in prolactin secretion via the hypothalamus. Such agents may act by altering the contents of catecholamines and PIF in the hypothalamus. Drugs and hormones may also have a direct effect upon pituitary prolactin secretion. It was the objective of the present investigation to determine the effects on growth of carcinogen-induced mammary tumors in rats of some drugs and hormones that previously have been demonstrated to alter prolactin secretion.

LITERATURE REVIEW

Hypothalamic control of prolactin secretion

During the 1950's the work of several researchers presented *in vivo* evidence for an inhibitory influence of the hypothalamus on anterior pituitary prolactin secretion (Desclin, 1950, 1956; Everett, 1954, 1956; Sanders and Rennels, 1957; Alloiteau, 1958; Boot *et al.*, 1959). Later, Pasteels (1961a,b) and Meites *et al.* (1961) showed an inhibition of prolactin secretion by the hypothalamus *in vitro*. Finally, a series of thorough and convincing experiments (Talwalker *et al.*, 1963) left little doubt regarding the validity of the concept of a specific "prolactin-inhibiting factor" (PIF) of hypothalamic origin. Other laboratories have confirmed this work (Danon *et al.*, 1963; Gala and Reece, 1964), and today it is firmly entrenched as a scientific fact. PIF is produced in the hypothalamus and is carried by way of the hypophysial portal circulation to the anterior pituitary gland where it exerts an inhibitory control over pituitary prolactin secretion.

When the anterior pituitary gland can escape hypothalamic control, the secretion of prolactin is increased (Meites *et al.*, 1963). Hypothalamic involvement is lost after specific median eminence lesions, pituitary stalk section, some central nervous system-depressant drugs, pituitary transplants, or *in vitro* culture. In all cases, after hypothalamic control is removed, prolactin secretion is increased. As a

result of the elevated prolactin concentrations, changes may occur in the mammary glands, ovaries, uterus, and vagina (Sulman, 1970).

The role of catecholamines

The hypothalamus contains a high content of norepinephrine and dopamine (Vogt, 1954; Laverty and Sharman, 1965). These two catecholamines are present in areas of the hypothalamus which have influence over the secretory activity of the anterior pituitary gland. Nerve terminal concentrations of norepinephrine are highest in the supraoptic, paraventricular, dorsomedial, and periventricular nuclei (Fuxe, 1965), while DA neuron cell bodies are concentrated in the arcuate and periventricular nuclei with their terminals in the median eminence (Fuxe et al., 1967). Evidence implicating these catecholamines as participants in control of anterior pituitary function has been accumulating for the last several decades. Everett (1964) has published a review which presents evidence supporting the possible involvement of the autonomic nervous system in regulation of gonadotropin secretions.

Changes in hypothalamic input cause fluctuations in catecholamine content. For example, lesions in the tegmentum of the mesencephalon, lateral hypothalamic area, or subthalamus lead to the disappearance of hypothalamic catecholamine terminals (Dahlstrom et al., 1964; Anden et al., 1965, 1966a,b). Stefano and Donoso (1967) showed by fluorometric procedures that changes in the functional state of the pituitary-gonad axis tend to affect noradrenergic neurons in the anterior hypothalamus. Norepinephrine concentrations appeared to be maximal during proestrus and minimal at estrus. After castration norepinephrine concentrations increased, but simultaneous treatment with estrogen

and progesterone led to a reduction in norepinephrine concentrations in the hypothalamus. Dopamine fluctuations occurred at the same time and were found to be inversely related to the changes in norepinephrine (Donoso and Stefano, 1967; Donoso et al., 1969). Other workers claim that α-methyl-para-tyrosine or other agents inhibiting tyrosine hydroxylase, an enzyme important in norepinephrine synthesis, lead to the disappearance of the effects of gonadectomy (Wurtman, 1970). Reis and Wurtman (1968) demonstrated that brain norepinephrine content undergoes diurnal variations. In addition, stress, hypophysectomy, pregnancy, and lactation have been associated with changes in catecholamine content in the hypothalamus (Fuxe and Hokfelt, 1969). Coppola (1969) reported an age-related increase in catecholamine pool size and turnover rate; the increase is accelerated after puberty. His work showed that an absence of ovarian steroids (for example, after oopherectomy) was followed by a marked increase in pool size and turnover rate. Steroid replacement therapy lowered catecholamine content. From these experiments he concluded that there was a strong indication of a reciprocal relationship between circulating gonadal steroid concentrations and hypothalamic catecholamine release and/or synthesis.

In order to elucidate the possible involvement of catecholamines in the hypothalamus-pituitary axis, drugs have been used to alter hypothalamic catecholamine content. Drugs that increase hypothalamic catecholamines tend to decrease serum prolactin concentrations and drugs that depress hypothalamic catecholamines tend to increase serum prolactin concentrations (Coppola et al., 1965, 1966; Van Maanen and Smelick, 1968; MacLeod et al., 1969). Kamberi et al. (1971) reported

that infusion of epinephrine into the third ventricle had no effect on prolactin release except at pharmacological levels; however, low doses of dopamine were effective in inhibiting prolactin release, presumably by increasing the release of PIF. An intracarotid injection of dopamine or epinephrine was followed by a decrease in anterior pituitary prolactin content but no change in the serum prolactin concentration (Lu et al., 1970). A single intraperitoneal injection of L-dihydroxyphenylalanine (L-dopa) was effective in significantly reducing the serum prolactin concentration (Lu and Meites, 1971; Wedig and Gay, 1970). Norepinephrine infused into the third ventricle inhibited PIF activity in the hypothalamus (Mittler and Meites, 1967), thereby increasing prolactin release. These four agents, dopamine, norepinephrine, epinephrine, and L-dopa presumably raise brain catecholamine content directly.

Other drugs are believed to inhibit catecholamine degradation and, thus, lead to an increase of hypothalamic catecholamines. The monoamine oxidase inhibitors Iproniazid, Pargyline, and Lilly compound 15461 have all been found to reduce serum prolactin concentrations (Lu and Meites, 1971).

On the other hand, reserpine (Coppola et al., 1965; Ratner et al., 1965; MacLeod et al., 1969; Lu et al., 1970), chlorpromazine (Lu et al., 1970), haloperidol (Janssen, 1967; Abuzzahab, 1971; Dickerman et al., in press) and related neuroleptic drugs cause a decrease in hypothalamic catecholamines in the hypothalamus and a significant increase in prolactin concentrations in the serum. Blockers of catecholamine-synthetic enzymes also cause an increase in serum

prolactin concentrations. Some of these are α-methyl-para-tyrosine, α-methyl-meta-tyrosine, and α-methyl-dopa (Fuxe and Hokfelt, 1969; Glowinski, 1970; Lu et al., 1970; Lu and Meites, 1971).

Thus, in summary, the hypothalamus exerts control over the secretory activity of the anterior pituitary gland and is known to contain a high content of catecholamines. Changes in the reproductive cycle produce changes in hypothalamic catecholamine content. Drugs able to increase or decrease catecholamine content of the hypothalamus are available. An increase in hypothalamic catecholamines results in increased PIF activity and decreased prolactin release, whereas a decrease in hypothalamic catecholamines results in decreased PIF activity and increased prolactin release. There is a strong indication that circulating gonadal steroids play an important role in the catecholamine fluctuations in the hypothalamus.

Direct effects of hormones and drugs

A number of hormones and drugs are capable of acting directly upon the adenohypophysis to influence prolactin release (see Meites et al., 1963). Of the gonadal, thyroid, and adrenal cortical hormones capable of stimulating prolactin release, estrogen appears to be the most notable. Employing the use of a highly sensitive radioimmunoassay, Chen and Meites (1970) treated different groups of ovariectomized rats with low, intermediate, or high doses of estradiol benzoate and found that all doses increased the serum and pituitary concentrations of prolactin. Estradiol benzoate implants in the median eminence also increase both serum and pituitary prolactin concentrations (Nagasawa et al., 1969). Removal of endogenous estrogen by ovariectomy and

adrenalectomy is followed by a decrease in prolactin concentrations (Pearson et al., 1969). Estrogen or a combination of estrogen and progesterone have been shown to increase serum prolactin concentrations indirectly by decreasing PIF in the hypothalamus (Ratner and Meites, 1964; Minaguchi and Meites, 1967). In addition to this indirect effect, estrogen also exerts a direct effect upon the anterior pituitary gland to enhance the secretion of prolactin (Nicoll and Meites, 1962; Ratner et al., 1963).

In contrast, certain ergot drugs are known to decrease the serum prolactin concentrations. Nagasawa and Meites (1970) showed that ergocornine decreased serum and pituitary prolactin concentrations. Further work had indicated that ergocornine probably acts directly upon the anterior pituitary gland (Malven and Hoge, 1971; Lu et al., 1971) and also indirectly by increasing the PIF content of the hypothalamus (Wuttke et al., 1971) to inhibit prolactin secretion. Zeilmaker and Carlsen (1962) administered ergocornine to lactating rats, resulting in a temporary inhibition of milk production. Ergocryptine, ergonovine, and several other ergot alkaloids have recently been found to reduce serum prolactin concentrations (Meites et al., unpublished). Quadri and Meites (1971) demonstrated the effectiveness of lysergic acid diethylamide (LSD) in preventing the prolactin peak in the rat during the afternoon of proestrus.

It is evident from the preceding that a number of agents normally present in the mammalian system interact to influence the synthesis and release of prolactin. In addition, there are a number of synthetic drugs capable of directly affecting the internal concentration of prolactin. By carefully experimenting with these hormones

and drugs, endocrinologists hope to learn more about the physiology of the pituitary and hypothalamus and to be able to correct malfunctions.

Short loop feedback

Meites and Sgouris (1953) first suggested that prolactin might feed back on the pituitary to inhibit prolactin secretion. Subsequent work by MacLeod et al. (1966) and Chen et al. (1967) showed that prolactin-secreting pituitary tumor transplants led to a decrease in hypothalamic PIF activity. This suggested that the feedback mechanisms acted via the hypothalamus. Later work by Clemens and Meites (1967, 1968) revealed that median eminence implants of prolactin also significantly decreased serum and pituitary prolactin concentrations as a result of increasing PIF in the hypothalamus. Transplantation of two or more anterior pituitaries or daily administration of exogenous prolactin to intact rats resulted in a decreased concentration of prolactin in the in situ pituitary (Welsch et al., 1968; Sinha and Tucker, 1968). Finally, using the sensitive prolactin radioimmunoassay, Voogt and Meites (1971) found that prolactin implants in the median eminence of pseudopregnant rats caused a decrease in the concentration of prolactin in the pituitary, termination of pseudopregnancy in most rats within three days, and failure of prolactin to reach normal concentrations in the serum in the subsequent estrus. The efforts of these various workers indicate that prolactin, indeed, has a profound inhibitory influence on its own secretion.

Mammary tumor induction, development, growth, and inhibition

The induction of mammary tumors in laboratory animals may be carried out by any of several methods. In mice mammary cancer may be elicited by treatment with estrogens or prolactin, or by pituitary transplants (Muhlbock and Boot, 1967; Boot, 1969). In rats the methods include estrogen treatment (Noble and Collip, 1941; Noble and Cutts, 1959; Huggins, 1965), exposure to radiation (Hamilton et al., 1954; Huggins and Fukunishi, 1963), and administration of aromatic chemical carcinogens--the last being the most effective and convenient means of producing experimental mammary tumors. These aromatic carcinogens include 2-acetaminofluorene (Wilson et al., 1941), methylcholanthrene (Shay et al., 1949), and dimethylbenzanthracene (Bachmann et al., 1938). Mammary adenocarcinoma induced by 7,12-dimethylbenzanthracene (DMBA) appears to be hormone-dependent (Pearson et al., 1969), and Huggins (1965) demonstrated a quick and simple method for the induction of mammary cancer of this type in 100% of the rats treated. Thus, researchers now have an experimental situation closely resembling hormone-dependent human breast cancer.

Much of the original work leading to an understanding of the hormonal relationships associated with breast cancer in humans has come from experimental surgical ablation. Thus, ovariectomy (Beatson, 1896), adrenalectomy (Fekete et al., 1941), orchidectomy (Farrow and Adair, 1942), and hypophysectomy (Luft and Olivecrona, 1953) were recommended as measures to be taken in cases of advanced breast cancer in humans. Along with hormone and drug treatments, these surgical procedures became weapons exhibiting varying degrees of effectiveness in the struggle against cancer.

Oopherectomy, in may cases, leads to a reduction in the size of carcinogen-induced mammary tumors, whereas estrogen treatment in ovariectomized rats previously treated with a carcinogen promotes growth of the tumors (Huggins et al., 1959). When rats are first ovariectomized and then treated with a carcinogen, no tumors develop (Huggins et al., 1961; Dao, 1962; Talwalker et al., 1964). However, if these rats are treated with estrogen or receive transplanted ovaries simultaneous with or shortly after administration of the carcinogen, tumors will appear although the incidence is lower than in intact rats treated with the carcinogen alone (Dao, 1962; Talwalker et al., 1964). These findings indicate that estrogen appears to be necessary for the development of mammary cancer in rats.

After hypophysectomy carcinogen-induced mammary tumors regress (Kim and Furth, 1960; Kim et al., 1960; Sterental et al., 1963). When, however, prolactin was administered to such rats, the tumors resumed growth rapidly, and many new tumors appeared (Pearson et al., 1969; Furth, 1961). Cessation of prolactin treatment resulted in a rapid regression of the tumors again. Estrogen administration to humans (Pearson and Ray, 1959) and rats (Sterental et al., 1963) could not produce an exacerbation of tumor growth after hypophysectomy. Such evidence strongly suggests that rat mammary cancer induced by DMBA is dependent on prolactin secretion from the anterior pituitary gland.

MacLeod et al. (1964) found that transplanted mammary tumors failed to grow in rats previously ovariectomized, even in the presence of a transplanted mammosomatotropic tumor. Daily treatments of estrogen and progesterone from the time of ovariectomy allowed growth of the

tumors. In a similar experiment Murota and Hollander (1971) delayed the estrogen and progesterone treatment for two months. Prolactin concentrations in the serum increased and the mammosomatotropic tumor continued to grow, but the transplanted mammary tumor failed to grow. However, one month after the onset of estrogen and progesterone treatment another mammary tumor was transplanted, and this time the tumor survived and began to grow promptly. These studies appear to indicate a requirement for ovarian stimulation of the mammary gland in order for mammary cancer to develop.

Recent work suggests that prolactin may play a major role in the development of mammary tumors, while estrogen is necessary to prepare the mammary gland for the action of prolactin. Talwalker et al. (1964) found that ovariectomized rats treated with DMBA did not develop mammary tumors. When, in addition, estrogen or a combination of prolactin and growth hormone were administered, some tumors developed, although not in 100% of the cases as with intact rats injected with DMBA. By experimentally increasing serum prolactin concentrations, Clemens et al. (1968) observed maintenance of tumor growth even after removal of the ovaries. The same researchers found similar results when they removed both ovaries and adrenals while inducing elevation of the serum prolactin concentrations (Welsch et al., 1969). Despite the fact that in both cases the tumors could be maintained only temporarily, the results indicate that prolactin plays an important role in tumor growth. At any rate it is evident that both prolactin and estrogen are required for the development of carcinogen-induced mammary tumors. Apparently prolactin and growth hormone are able to promote tumor development in the mammary

gland without the ovaries, but as mentioned previously estrogens were unable to promote mammary cancer in the absence of the pituitary gland.

Experimental manipulations on pituitary prolactin secretion should exert a profound influence upon mammary cancers. Indeed, Welsch et al. (1970a) found a marked increase in the incidence of spontaneous mammary tumors in rats bearing multiple pituitary grafts. Bilateral lesions of the median eminence evoked similar results (Clemens et al., 1968; Welsch et al., 1969, 1970b). Both experimental situations are known to increase the serum prolactin concentration. In addition, it has been reported that certain drugs known to enhance circulating prolactin concentrations can promote development of DMBA-induced mammary tumors. Both perphenazine (Pearson et al., 1969) and reserpine (Welsch and Meites, 1968) yield such a response. These findings constitute further evidence for the major role of prolactin in promotion of DMBA-induced mammary tumors in rats.

On the other hand, certain drugs that inhibit prolactin secretion have been demonstrated to decrease growth and development of mammary tumors. Two ergot drugs which were found to suppress mammary cancer are ergocornine (Nagasawa and Meites, 1970; Yanai and Nagasawa, 1970; Cassell et al., 1971) and ergocryptine (Heuson et al., 1970; Cassell et al., 1971).

The role of estrogen is of great importance in the interrelationships of endocrine organs and their influence upon the mammary gland and the development of tumors. Estrogen administration in high, intermediate, or low doses causes an increase in serum and pituitary prolactin concentrations, while ovariectomy and adrenalectomy are

followed by a decrease in serum prolactin concentrations in the rat. Thus, tumor growth may be affected by variations of the estrogen titer in the blood. Nagasawa et al. (1969) found that estrogen implanted in the median eminence of rats with DMBA-induced mammary tumors increased serum and pituitary prolactin concentrations and growth of mammary tumors. In another experiment, Welsch et al. (1969) reported that ovariectomy at the time of median eminence lesion was followed by an initial stimulation of mammary tumor development with a subsequent regression. The stimulation is presumably due to the elevated prolactin concentrations in the serum associated with the median eminence lesion; however, regression of the tumors suggests that removal of the ovaries is responsible for the latter phenomenon. Thus, it appears that, although prolactin may be the major hormone promoting mammary tumor growth, estrogen may affect the growth and development of mammary tumors in rats.

Estrogen has been used for years in humans to keep breast cancer in check (Haddow, 1935; Landau et al., 1962). Meites (in press) found that high doses of estrogen (20 µg) caused mammary tumor regression in rats despite the fact that prolactin concentrations were elevated. Male sex hormones have also been discovered to be effective against mammary cancers (Lacassagne, 1939; Loeser, 1941; Council on Pharmacy and Chemistry, 1951). Others have shown that an estrogen-progesterone combination (Huggins and Yang, 1962; Landau et al., 1962) or potent synthetic estrogens (Haddow et al., 1944; Nathanson, 1946) may lead to regression of breast cancer.

Although high or low serum prolactin concentrations may be a major influence on the promotion or regression of mammary tumors in rats, there are other factors which may play a less significant role but exert considerable influence upon the induction, growth, and development of mammary cancer. Therefore, the purpose of the experimental work in this thesis was to attempt to elucidate some of the factors involved in control of mammary tumor growth in rats, and hopefully, to provide some new approaches for treating human breast cancer.

MATERIALS AND METHODS

All animals used in these experiments were virgin female Sprague-Dawley rats obtained from Spartan Research Animals, Inc., Haslett, Mich. The animals were maintained on a regular lighting schedule of 14 hours of light per day. Temperature was held constant at 24 ± 1° C, and food (Wayne Lab Blox, Allied Mills, Chicago, Ill.) and tap water were given ad libitum. In addition, dietary supplements of carrots and oranges were supplied weekly.

At 55 days of age intact rats were administered a single intravenous injection into the tail vein of a lipid emulsion containing 5 mg of 7,12-dimethylbenzanthracene (DMBA) (The Upjohn Co., Kalamazoo, Mich.) after the method of Huggins (1965). Palpable mammary tumors were present within 1-3 months after DMBA administration in 100% of the animals. The rats were then divided into groups of equal size with efforts for uniformity of tumor incidence and mean tumor diameter among the groups. With the rats placed under light ether anasthesia, total tumor number per rat, the largest diameter per tumor as measured by calipers, and body weights in grams were recorded at the beginning of each experiment. These measurements continued at regular intervals after the onset of treatment. Further details of the treatments are given under each separate experiment.

All data were analyzed by comparing regression lines through an analysis of covariance.

EXPERIMENTAL

Experiment I: Effects of Estradiol Benzoate and Prolactin on Growth of Carcinogen-induced Mammary Tumors

Objectives

The purpose of the first experiment was to learn more about the mechanism whereby large doses of estrogen inhibit mammary tumor development. Prolactin and estrogen can promote mammary tumor development and growth. Small doses of estrogen can promote mammary tumor development and growth, whereas large doses are known to inhibit mammary tumor growth. Large doses of estrogen are commonly used in the treatment of breast cancer in women. Likewise, such large doses of estrogen inhibit mammary adenocarcinoma in rats and other experimental animals. Nevertheless, estrogen at high, intermediate, or low doses increases serum prolactin concentrations and has never been observed to decrease the prolactin concentration in the serum. Therefore, large doses of estrogen do not appear to inhibit mammary tumor growth by inhibiting prolactin secretion. Thus, the objective of this investigation was to determine how large doses of estrogen could inhibit mammary tumor growth in rats.

Procedure

All treatments were administered via subcutaneous injection daily for 20 days. Treatments were as follows:

GROUP 1: 0.2 ml of corn oil (controls)

GROUP 2: 20 µg of estradiol benzoate (Nutritional Biochemicals Corp., Cleveland, Ohio) in 0.2 ml of corn oil

GROUP 3: 20 µg of estradiol benzoate in 0.2 ml of corn oil + 1 mg of ovine prolactin (NIH-P-S-8, 28 IU/mg, National Pituitary Agency, NIH) in 0.2 ml of 0.9% saline

Largest tumor diameter, number of tumors per rat, and body weights in grams were measured every 5 days during the 20 days of treatment.

Results

Mean tumor diameter of the control group increased from 70 ± 12 mm to 107 ± 16 mm and mean number of tumors per rat increased from 2.4 ± 0.4 to 3.4 ± 0.7 during the 20 days of treatment (Table I). The group receiving estradiol benzoate exhibited a decrease in mean tumor diameter from 131 ± 21 mm to 126 ± 30 mm which was significantly different ($p < .01$) from the control group. Likewise, the mean tumor incidence decreased from 2.5 ± 0.4 to 1.7 ± 0.3 and this was also significantly different ($p < .01$) from the control group (see Figure I).

The group administered estradiol benzoate and prolactin showed an increase in mean tumor diameter from 95 ± 13 mm to 142 ± 16 mm and in mean tumor incidence from 2.9 ± 0.3 to 3.2 ± 0.4. Neither was different from the control group. It appears that the ovine prolactin overcomes the effect of the estradiol benzoate in inhibiting mammary tumor growth.

Table I. Effects of Estradiol Benzoate (EB) and Prolactin on Growth of Carcinogen-induced Mammary Tumors

TREATMENT (No. of Rats)	MEAN BODY WT. (gms)		MEAN TUMOR DIAMETER (mm) ± S.E. (Mean No. of Tumors Per Rat ± S.E.)				
	initial	final	onset	5 days	10 days	15 days	20 days
Controls (12)	269	283	70 ± 12	81 ± 13	91 ± 13	90 ± 1.6	107 ± 16
			(2.4 ± 0.4)	(2.8 ± 0.3)	(3.2 ± 0.5)	(2.9 ± 0.6)	(3.4 ± 0.7)
EB (10)	272	283	131 ± 21	141 ± 25	137 ± 25	128 ± 27	126 ± 30
			(2.5 ± 0.4)	(2.8 ± 0.5)	(2.6 ± 0.5)	(1.9 ± 0.3)	(1.7 ± 0.3)
EB + Prolactin (10)	270	286	95 ± 13	106 ± 13	125 ± 17	131 ± 18	142 ± 16
			(2.9 ± 0.3)	(3.0 ± 0.4)	(3.4 ± 0.5)	(3.1 ± 0.5)	(3.2 ± 0.4)

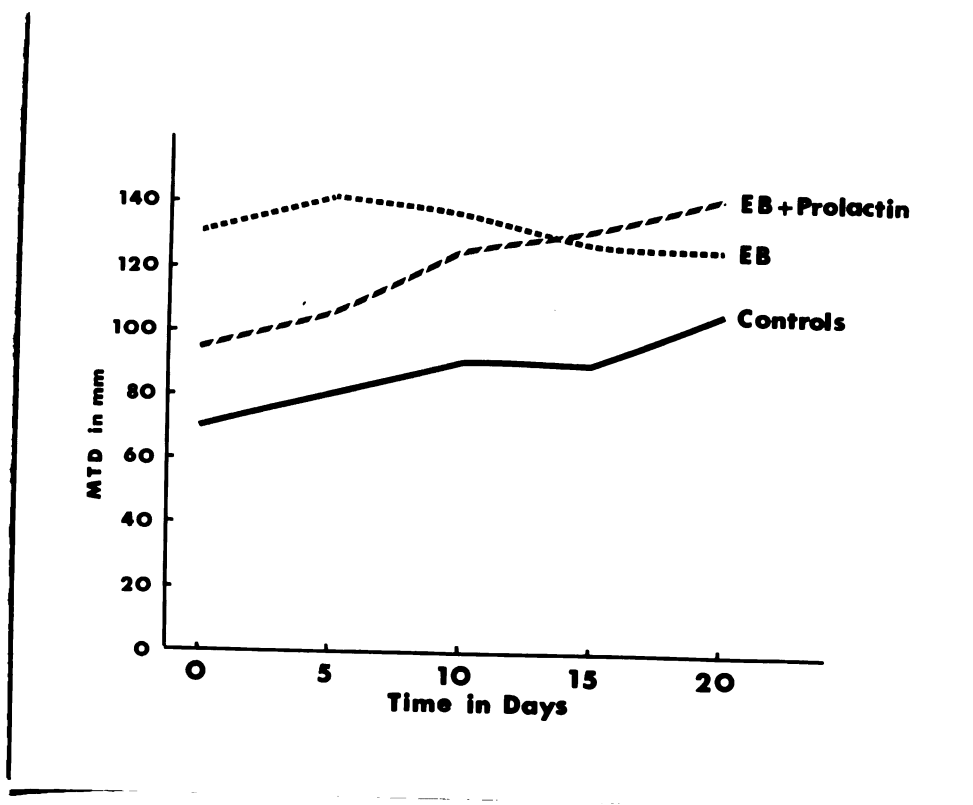

Figure I. Effects of Estradiol Benzoate and Prolactin on Growth of Carcinogen-induced Mammary Tumors. MTD = mean tumor diameter. Treatments were: 20 µg of estradiol benzoate (EB) alone or with 1 mg of ovine prolactin; controls received corn oil.

Experiment II: Effects on Mammary Tumor Growth of Prolactin, Testosterone, and Drugs that Alter Hypothalamic Catecholamines

Objectives

This investigation was undertaken in order to explain the effects of experimentally increasing serum prolactin concentration, serum testosterone concentration, or brain catecholamine content on mammary tumor growth. Prolactin stimulates mammary tumor growth. Testosterone can directly inhibit mammary tumor growth, while increasing the catecholamine content in the hypothalamus inhibits prolactin secretion.

Procedure

All treatments were administered via subcutaneous injection daily for 3 weeks. They were as follows:

GROUP 1: 0.3 ml of 0.9% saline (controls)

GROUP 2: 10 mg of Iproniazid phosphate (Hoffman-LaRoche, Inc., Nutley, N.J.) in 0.3 ml of 0.9% saline

GROUP 3: 15 mg of Pargyline hydrochloride (Abbott Laboratories, North Chicago, Ill.) in 0.3 ml of 0.9% saline

GROUP 4: 10 mg of Levodopa dihydroxyphenylalanine (L-dopa) (Nutritional Biochemicals Corp., Cleveland, Ohio) in 0.34 ml final volume of a combination of 0.5 N HCl brought to pH 6.5 by 0.5 N NaOH

GROUP 5: 5 mg of testosterone propionate (Nutritional Biochemicals Corp., Cleveland, Ohio) in 0.2 ml corn oil

GROUP 6: 1 mg of ovine prolactin (NIH-P-S-8, 28 IU/mg, National Pituitary Agency, NIH) in 0.2 ml of 0.9% saline

GROUP 7: 5 mg of testosterone propionate in 0.2 ml of corn oil + 1 mg of ovine prolactin in 0.2 ml of 0.9% saline

After one week of treatment an androgen with more anabolic potency was used to replace testosterone propionate. Five mg of 11β-hydroxy-17-methyltestosterone in 0.2 ml of corn oil was substituted for the duration of the experiment.

Measurements of body weights in grams, mammary tumor diameters in mm, and mammary tumor incidence were recorded every 7 days. Treatments lasted for a period of 3 weeks.

Results

Mean tumor diameter of the control group increased from 35 ± 11 mm to 59 ± 22 mm and mean tumor incidence increased from 3.1 ± 1.1 to 3.8 ± 1.4 during the 3 weeks of treatment (Table II). The prolactin group exhibited an increase in both mean tumor diameter from 40 ± 12 mm to 118 ± 22 mm and the mean number of tumors per rat from 3.0 ± 1.0 to 8.6 ± 1.5. When compared to the control group, the mean tumor diameter and mean tumor incidence of the prolactin group showed a significant ($p < .001$) increase (see Figure II).

On the other hand, Pargyline, with an initial mean tumor diameter of 45 ± 13 mm and a final mean tumor diameter of 27 ± 9 mm, and Iproniazid, with an initial mean tumor diameter of 37 ± 16 mm and a final mean tumor diameter of 39 ± 9 mm, were both significantly different ($p < .01$) from the control group in their effect on mean tumor diameter. Although the rats in the Iproniazid group showed no change in mean tumor incidence which was 3.8 ± 1.1 at the beginning of the experiment and 4.0 ± 1.1 at the end, the Pargyline group exhibited a decrease in mean tumor incidence from the original 3.4 ± 0.8 to 2.3 ± 0.6 which was significant ($p < .01$) when compared to the controls.

Table II. Effects on Mammary Tumor Growth of Prolactin, Testosterone, and Drugs that Alter Hypothalamic Catecholamines

TREATMENT (No. of Rats)	MEAN BODY WT. (gms)		MEAN TUMOR DIAMETER (mm) ± S.E. (Mean No. of Tumors Per Rat ± S.E.)			
	initial	final	onset	1 week	2 weeks	3 weeks
Controls (9)	285	306	35 ± 11 (3.1 ± 1.1)	46 ± 16 (3.4 ± 1.4)	53 ± 19 (3.9 ± 1.5)	59 ± 22 (3.8 ± 1.4)
Prolactin (7)	287	298	40 ± 12 (3.0 ± 1.0)	63 ± 15 (4.7 ± 1.4)	89 ± 20 (5.9 ± 1.3)	118 ± 22 (8.6 ± 1.5)
Androgen (6)	298	304	41 ± 9 (3.7 ± 0.8)	52 ± 11 (4.2 ± 0.8)	57 ± 11 (4.2 ± 0.8)	58 ± 19 (4.0 ± 0.8)
Androgen & Prolactin (7)	299	319	40 ± 19 (3.0 ± 0.6)	60 ± 10 (3.7 ± 0.7)	67 ± 10 (3.9 ± 0.8)	69 ± 10 (3.7 ± 0.7)
L-dopa (6)	288	294	43 ± 17 (3.0 ± 1.2)	53 ± 19 (3.2 ± 1.4)	57 ± 19 (3.3 ± 1.4)	61 ± 21 (3.7 ± 1.5)
Iproniazid (4)	276	249	37 ± 16 (3.8 ± 1.1)	49 ± 17 (4.3 ± 1.4)	40 ± 9 (4.3 ± 1.5)	39 ± 9 (4.0 ± 1.1)
Pargyline (7)	288	258	45 ± 13 (3.4 ± 0.8)	39 ± 12 (3.0 ± 0.7)	32 ± 11 (2.9 ± 0.6)	27 ± 9 (2.3 ± 0.6)

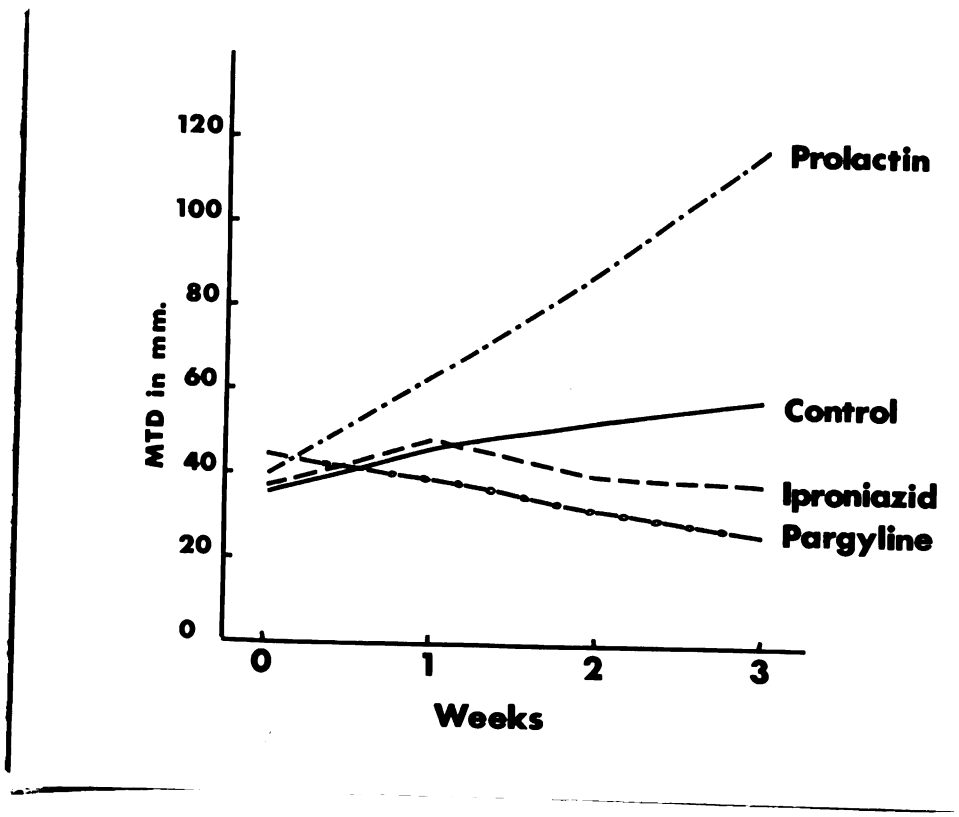

Figure II. Effects on Mammary Tumor Growth of Prolactin, Testosterone, and Drugs that Alter Hypothalamic Catecholamines. MTD = mean tumor diameter. Treatments were: 1 mg ovine prolactin, 15 mg Iproniazid (reduced to 5 mg), 10 mg Pargyline (reduced to 5 mg), controls received saline.

L-dopa and androgen treatment were ineffective in inhibiting mammary tumor growth. Mean tumor diameters and mean tumor incidences increased from 43 ± 17 mm and 3.0 ± 1.2 to 61 ± 21 mm and 3.7 ± 1.5, respectively, for L-dopa and from 41 ± 9 mm and 3.7 ± 0.8 tumors per rat to 58 ± 19 mm and 4.0 ± 0.8 tumors per rat for the androgen-treated group (see Figure III).

An increase in mean tumor diameter from 40 ± 19 mm to 69 ± 10 mm and an increase in the mean number of tumors per rat from 3.0 ± 0.6 to 3.7 ± 0.7 during the 3 weeks of treatment for the group treated with androgen and prolactin together were not different from the increase in the same measurements in the control group. Likewise, there is no difference between this group and the group treated with androgen alone. However, when the group treated with androgen and prolactin is compared to the group receiving prolactin alone there is a significant inhibition ($p < .01$) of mammary tumor growth in the former. This indicates that the androgen 11β-hydroxy-17-methyltestosterone can overcome the mammary tumor growth-stimulating effects of ovine prolactin.

In spite of their respective effects upon prolactin secretion and the mammary glands, L-dopa and the androgen did not inhibit mammary tumor growth. This may be explained by the facts that L-dopa acts rapidly but has a short duration, while the androgen is not as potent as those used clinically.

Both Iproniazid and Pargyline caused hyperactivity in the rats. At the end of the first week the dose of Iproniazid was decreased by half, and by the end of the second week both drugs were reduced to 5 mg daily per rat. Despite the reduction in dose, animals in both groups lost weight and several died.

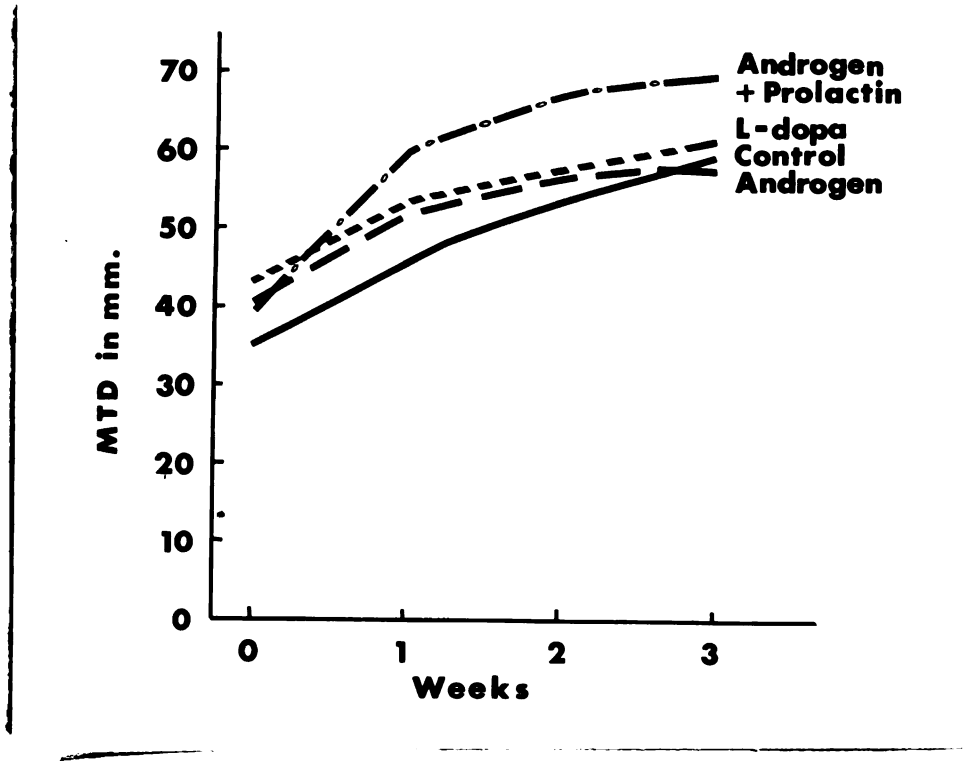

Figure III. Effects on Mammary Tumor Growth of Prolactin, Testosterone, and Drugs that Alter Hypothalamic Catecholamines. MTD = mean tumor diameter. Treatments were: 5 mg of androgen alone or with 1 mg of ovine prolactin, 10 mg L-dopa, controls received saline.

Experiment III. Effects on Mammary Tumor Growth of Ergots, Haloperidol, and Pargyline

Objectives

The purpose of the final experiment was to test the effects on mammary tumor growth of the two ergot drugs ergonovine and lysergic acid diethylamide (LSD) and the tranquilizing drug haloperidol, which inhibits hypothalamic catecholamine activity. A reduced dose of Pargyline was tried with the aim of inhibiting mammary tumor growth without causing a loss of body weight or a hyperactivity in the animals.

Procedure

Rats in each group were administered a single, daily injection subcutaneously as listed below:

GROUP 1: 0.3 ml of corn oil (controls)

GROUP 2: 150 µg of haloperidol (McNeill Laboratories, Inc., Fort Washington, Penn.) in 0.3 ml of corn oil emulsion

GROUP 3: 1.5 µg the 1st week, 3.0 µg the 2nd week, 6.0 µg the 3rd week of LSD (courtesy of Dr. T. M. Brody, Chairman of the Department of Pharmacology, Michigan State University, East Lansing, Mich.) in 0.3 ml of a saline-corn oil mixture (1:1)

GROUP 4: 1.8 mg the 1st and 2nd weeks, 3.6 mg the 3rd week of ergonovine (Eli Lilly and Co., Indianapolis, Ind.) in 0.3 ml of 0.9% saline

GROUP 5: 6.0 mg of Pargyline hydrochloride (Abbott Laboratories, North Chicago, Ill.) in 0.3 ml of 0.9% saline

Treatments lasted for a period of 3 weeks and measurements of the largest tumor diameter, tumor incidence, and body weights in grams were recorded every 7 days.

Results

Mean tumor diameter of the control group increased from 51 ± 16 mm to 82 ± 24 mm and mean tumor incidence from 3.6 ± 1.1 to 6.0 ± 1.3 during the 3 weeks of treatment (Table III). The haloperidol group exhibited an increase in both tumor diameter from 29 ± 10 mm to 131 ± 31 mm and the mean number of tumors per rat from 2.7 ± 0.9 to 8.5 ± 1.3. When compared to the control group, the mean tumor diameter and the mean tumor incidence of the haloperiodol group showed a significant increase ($p < .01$) (see Figure IV).

On the other hand, Pargyline, with an initial mean tumor diameter of 65 ± 20 mm and a final mean tumor diameter of 43 ± 17 mm, significantly inhibited mammary tumor growth ($p < .01$). Likewise, there was a significant decrease ($p < .01$) in the mean number of tumors per rat from 4.3 ± 0.9 to 3.3 ± 0.7 when compared to the control group.

Mean tumor diameters and mean tumor incidences increased in the groups receiving ergonovine, from 55 ± 18 mm to 68 ± 20 mm and 3.9 ± 1.2 to 4.6 ± 1.3, respectively, and LSD, from 49 ± 18 mm to 59 ± 16 mm and 4.0 ± 1.2 to 4.6 ± 1.3, but neither group exhibited a significant difference when compared to the control group. However, the final dose of LSD (6 μg) appears to have an effect on the mammary tumors; perhaps this dose would inhibit mammary tumor growth if administered for 3 weeks.

Rats did not appear to be harmed by the reduced amount of Pargyline although they still lost weight. None of the other groups exhibited any unusual reactions to treatment. The animals receiving LSD seemed generally more docile than the rats in the other groups.

Table III. Effects on Mammary Tumor Growth of Ergots, Haloperidol, and Pargyline

TREATMENT (No. of Rats)	MEAN BODY WT. (gms)		MEAN TUMOR DIAMETER (mm) S.E. (Mean No. of Tumors Per Rat S.E.)			
	initial	final	onset	1 week	2 weeks	3 weeks
Controls (8)	275	279	51 ± 16 (3.6 ± 1.1)	58 ± 16 (4.3 ± 1.3)	68 ± 17 (4.9 ± 1.2)	82 ± 24 (6.0 ± 1.3)
Ergonovine (7)	299	281	55 ± 18 (3.9 ± 1.2)	61 ± 20 (4.0 ± 1.1)	66 ± 19 (4.0 ± 1.2)	68 ± 20 (4.6 ± 1.3)
Lysergic acid diethylamide (LSD) (7)	277	265	49 ± 18 (4.0 ± 1.2)	59 ± 20 (4.6 ± 1.3)	63 ± 21 (4.6 ± 1.5)	59 ± 16 (4.6 ± 1.3)
Pargyline (8)	281	253	65 ± 20 (4.3 ± 0.9)	56 ± 18 (4.3 ± 0.8)	45 ± 17 (3.3 ± 0.8)	43 ± 17 (3.3 ± 0.7)
Haloperidol (6)	293	287	29 ± 10 (2.7 ± 0.9)	52 ± 12 (4.7 ± 1.3)	79 ± 17 (6.0 ± 1.4)	131 ± 31 (8.5 ± 1.3)

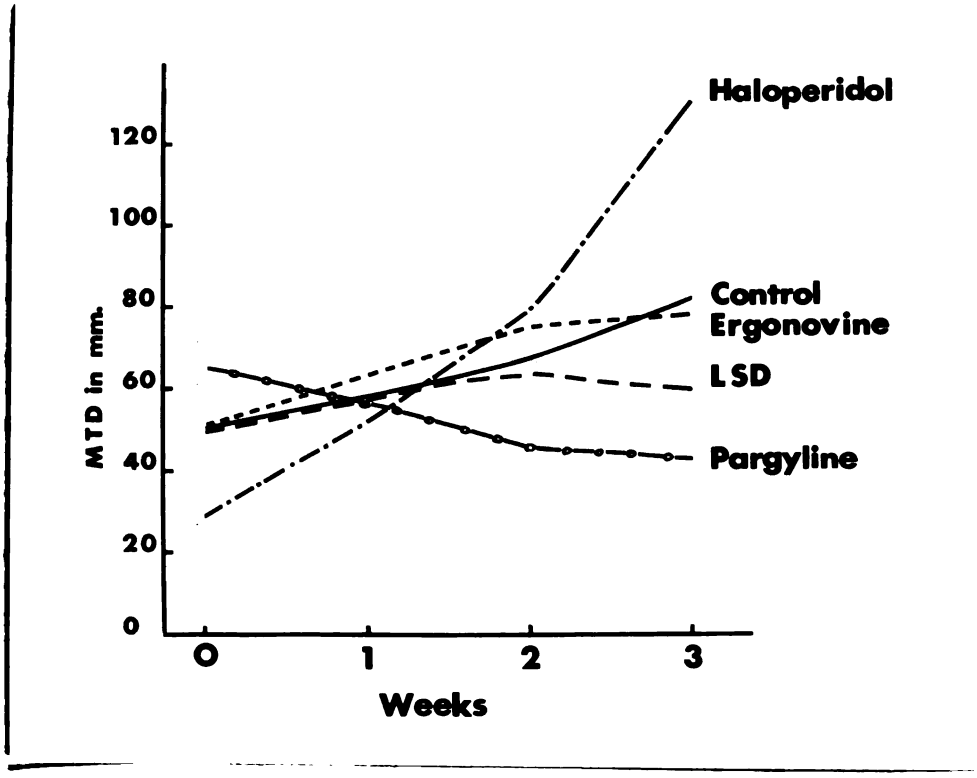

Figure IV. Effects on Mammary Tumor Growth of Ergots, Haloperidol, and Pargyline. MTD = mean tumor diameter. Treatments were: 150 µg haloperidol, 1.5-6.0 µg LSD, 1.8-3.6 mg ergonovine, and 6 mg Pargyline; controls received corn oil.

DISCUSSION

Prolactin appears to be the most important hormone related to the promotion of mammary tumor growth in rats (Furth, 1961, 1962; Meites and Nicoll, 1966; Pearson et al., 1969; Welsch et al., 1970a,b). This research involved attempts to alter carcinogen-induced mammary tumor growth in rats by indirect manipulation of prolactin concentration through alteration of hypothalamic control of prolactin secretion. The DMBA-induced mammary tumor and human breast cancer resemble each other in two respects--their common origin in ductal tissue and their responsiveness to hormone treatment. The types of drugs employed in these experiments have all been shown to affect circulating prolactin concentrations, while the steroid hormones also have direct effects upon the mammary glands.

An attempt was made to determine the mechanism by which large doses of estrogen inhibit mammary cancer growth. The results of this work indicate that large doses of estrogen interfere with the peripheral action of prolactin on mammary tumor growth. Large doses of estrogen have been used by clinicians and researchers to inhibit breast cancer in women and experimental animals. However, it was found that all doses stimulate prolactin release from the anterior pituitary gland (Chen and Meites, 1970). Therefore, the mechanism of action of large doses of estrogen in inhibiting mammary tumor growth cannot be explained by the inhibition of prolactin secretion. Meites and Sgouris (1954) found

that ovarian steroids appeared to prevent prolactin from exerting its full influence upon the mammary gland. The results of the first experiment suggest that this may be the mechanism by which estradiol benzoate inhibits mammary tumor growth.

Nagasawa and Meites (unpublished), using several dose levels of estradiol benzoate, found that daily injections of a dose of 20 μg was most effective in inhibiting mammary tumor growth in rats. The results of Experiment I show that this dose of estradiol benzoate was effective in preventing the increase of mean tumor diameter and mean number of tumors per rat found in the control group. Increasing the amount of prolactin in the serum by daily injections of exogenous NIH ovine prolactin (1 mg) overcame the inhibition by estradiol benzoate, and mean tumor diameter and mean tumor incidence were no different from those of the control group. These findings suggest that large doses of estrogen interfere with the peripheral action of prolactin on the mammary tissue and, thereby, inhibit growth of the mammary adenocarcinoma. Further research is required in order to determine the biochemical mechanisms involved in this interaction between estrogen and prolactin.

Androgens have also been used in the treatment of mammary cancer with effectiveness in 20% or more of the cases (Rosoff, 1960; Calabresi and Parks, 1970). In Experiment II testosterone propionate was found to be ineffective during the first week of treatment and a more potent anabolic androgen, 11β-hydroxy-17-methyltestosterone, was substituted. However, this hormone also failed to inhibit mammary tumor growth, although when given at the same time as prolactin, 11β-hydroxy-17-methyltestosterone prevented the significant increase in mean tumor

diameter found in the group receiving prolactin alone. Since clinical studies indicate that improvements in the status of patients with mammary cancers are very slow with androgen treatment, it would appear that the time span of this experiment was not sufficient for the effects of the androgen treatment to be manifested. However, the principal reason for the ineffectiveness of these two androgens is probably that they were not potent enough in androgenic activity.

In rats given prolactin there can be no doubt that there was a significant stimulatory effect upon the growth of mammary tumors. There was nearly a threefold increase in both mean tumor diameter and tumor incidence in three weeks. In addition, the effects of haloperidol in Experiment III are equally marked. Haloperidol has been found to stimulate an increase in serum prolactin concentration of greater than elevenfold in rats (Dickerman et al., in press). It was shown to deplete the hypothalamus of catecholamine and PIF activities. Thus, sufficient increase of circulating prolactin concentrations, either by injection of prolactin or indirectly by administering haloperidol to rats with DMBA-induced mammary tumors, promoted a powerful stimulation of mammary tumor growth. These findings lend strong credence to the concept that prolactin plays a major role in stimulating growth of carcinogen-induced mammary tumors in rats.

The remaining drugs in Experiment II, L-dopa, Iproniazid, and Pargyline, were employed in efforts to reduce the circulating concentration of prolactin and, in so doing, to prevent promotion of mammary tumor growth by prolactin. These three drugs are believed to increase the content of brain catecholamines, and they have been found to

decrease serum prolactin concentrations in rats (Lu and Meites, 1971). Both Iproniazid and Pargyline, inhibitors of catecholamine degradation, inhibited mammary tumor growth. L-dopa, a precursor in the biosynthesis of dopamine and norepinephrine, failed to inhibit mammary tumor growth in this experiment. Lu and Meites (1971) injected a similar dose of L-dopa intraperitoneally and found that it reduced serum prolactin concentrations for up to two hours after injection. It is possible, therefore, that more frequent treatment with L-dopa would have inhibited mammary tumor growth. The results of injecting two agents, Iproniazid and Pargyline, known to inhibit prolactin secretion into rats with DMBA-induced mammary tumors tend to support the concept used as the basis for this work.

Neither of the ergot drugs, ergonovine and LSD, inhibited mammary tumor growth. Ergot derivatives inhibit prolactin secretion. Quadri and Meites (1971) found that LSD inhibits the prolactin peak during proestrus. The fact that these two drugs were not effective in the inhibition of mammary tumor growth may be explained by the fact that the doses used were not high enough. The dose of ergonovine was doubled late in the second week of treatment and LSD doses were doubled at the end of weeks one and two. Both drugs appeared to be more effective in preventing mammary tumor growth during the third week of treatment. Previously, two other ergot drugs, ergocornine and ergocryptine, were shown to be effective in inhibiting mammary tumor growth in rats (Cassell et al., 1971; Nagasawa and Meites, 1970).

After termination of the treatments the growth of mammary tumors in the control group continued at the same rate. Although not recorded

in the present data, the trend for tumors whose growth had been inhibited by drugs earlier showed an increase in growth subsequent to termination of treatment. Those whose growth was previously stimulated (i.e., the prolactin and haloperidol groups) subsequently exhibited a decrease in the mammary tumor growth rate. A similar observation has been made by others (Cassell, 1971; Quadri and Meites, unpublished).

SUMMARY AND CONCLUSIONS

The effects of hormones and drugs on carcinogen-induced mammary tumors in rats were investigated in order to test further the current hypothesis that prolactin plays a major role in the promotion of mammary tumor growth. Some of the drugs used are known to alter catecholamine content of the hypothalamus and also to alter the secretion of prolactin. All agents employed in these experiments influenced prolactin and thereby caused inhibition or stimulation of mammary tumor growth.

1. EB administered daily in doses of 20 μg for 20 days completely inhibited growth of mammary tumors induced by DMBA, whereas mammary tumors in the control group increased by about 50% in mean tumor diameter. When 1 mg of ovine prolactin was administered simultaneous to 20 μg of EB, the inhibitory effect of EB was overcome completely and mammary tumor diameters increased about 50%. The inhibitory action of EB upon mammary tumor growth appears to be exerted via inhibition of the peripheral action of prolactin on the mammary gland. Administration of exogenous prolactin (1 mg) overcomes this inhibition by "flooding" the mammary tissue with prolactin.

2. Ovine prolactin (1 mg) injected daily for 3 weeks significantly increased growth of mammary tumors. Androgen treatment (5 mg of testosterone propionate for 1 week, followed by 5 mg of 11β-hydroxy-17-methyltestosterone for 2 weeks) had no effect upon mammary tumor growth. L-dopa (10 mg) for 3 weeks had no effect upon mammary tumor growth.

Both Iproniazid (15 mg daily, reduced to 5 mg) and Pargyline (10 mg daily, reduced to 5 mg) significantly inhibited mammary tumor growth. The dose of L-dopa may not have been sufficient to reduce prolactin secretion long enough to influence mammary tumor growth. The androgens were given in insufficient doses to inhibit growth of mammary tumors.

3. Daily injections of haloperidol (150 µg) for 3 weeks enhanced growth of DMBA-induced mammary tumors significantly. LSD (6 µg) and ergonovine (3.6 mg) appeared to have an inhibitory effect on mammary tumor growth, while Pargyline (6 mg) completely inhibited mammary tumor growth.

Haloperidol has been reported to decrease hypothalamic catecholamines, thereby increasing prolactin release from the anterior pituitary gland. Pargyline and Iproniazid interfere with prolactin release by increasing hypothalamic catecholamine and prolactin inhibiting factor (PIF) contents. LSD and ergonovine are believed to act directly on the anterior pituitary gland to inhibit prolactin secretion. This investigation supports the theory that prolactin is the major influence in the promotion of mammary tumor growth in rats.

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