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Inhibition of Estradiol-Induced Intranuclear Inclusions by an Anti-Estrogen (Nafoxidine) in Mammotrophs of the Mongolian Gerbil[1] (38682)

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Nakayama and Nickerson (1) have described intranuclear inclusions in mammotrophs of the Mongolian gerbil. These structures are found exclusively in the anterior pituitary gland of female gerbils and not in the male or in newborn animals of either sex. Estradiol increases the number of inclusions in females and induces these structures in mammotrophs of male gerbils (2). It was the purpose of the present study to determine whether an anti-estrogen (nafoxidine) (3) would modify the induction of inclusions in male gerbils.

Materials and Methods. Twenty male Mongolian gerbils (*Meriones unguiculatus*) weighing 52 ± 3 g were obtained from Tumblebrook Farms, North Brookfield, MA. Animals were caged individually and maintained at constant temperature (22.5°) and humidity in a Hot Pack Environmental Chamber. Gerbils were given free access to lab chow and tap water. Gerbils were divided equally into four groups: group 1 were controls and received sesame oil, group 2 received 0.5 mg nafoxidine hydrochloride, U-11,100A (kindly supplied by Upjohn Pharmaceuticals, Kalamazoo, MI), group 3 received 10 μg estradiol benzoate in sesame oil (Scherring Corporation, Bloomfield, NJ), and group 4 received 0.5 mg nafoxidine and 10 μg estradiol benzoate. Estradiol or sesame oil were injected im in a volume of 0.1 cm whereas 0.1 cm nafoxidine in 0.9% saline was injected sc. Injections of inhibitor for groups 2 and 4 were begun 1 wk before estrogen and then estrogen was given for 14 days; animals were sacrificed on the 21st day, 3 hr after the last injection.

The pituitary gland was removed quickly and fixed in 3% purified glutaraldehyde (Ladd Research Industries, Burlington, Vermont) buffered to pH 7.4 with 0.1 *M* phosphate. Tissues were processed and inclusions were counted in 1 μm toluidine blue stained sections as reported previously (2).

Results. No intranuclear inclusions were observed in 1 μm sections from control injected (group 1) or with animals receiving the inhibitor (group 2). However, with estrogen (group 3), 2.58 ± 0.10 inclusions were observed per high power field. Inhibitor plus estradiol (group 4) caused an inhibition in the inclusions (0.34 ± 0.04 intranuclear inclusions per field).

Estrogen promoted the proliferation of rough endoplasmic reticulum and the development of a hypertrophic Golgi apparatus in mammotrophs. It is of special interest that with group 4, nafoxidine did not prevent the estradiol-induced hypertrophy of rough endoplasmic reticulum and Golgi apparatus (Fig. 1). Vacuolar and membranous (Fig. 1) types of inclusions were identifiable. Mammotrophs from control or nafoxidine-injected animals showed no inclusions by electron microscopy nor changes in ultrastructure and were identical to controls described previously (1).

Discussion. The development of intranuclear inclusions was inhibited by nafoxidine; nafoxidine is an anti-estrogen which presumably blocks the combination of estrogen with a cytoplasmic receptor and therefore the entry of estrogen–receptor into the nucleus is blocked (4). Thus in the pituitary gland of the gerbil, combination of estrogen with receptor is at least in part required for induction of inclusions. A small number of inclusions were observed in estrogen–inhibitor treated animals. Although injections were given daily, injection may well not maintain a consistently high blood level of the inhibi-

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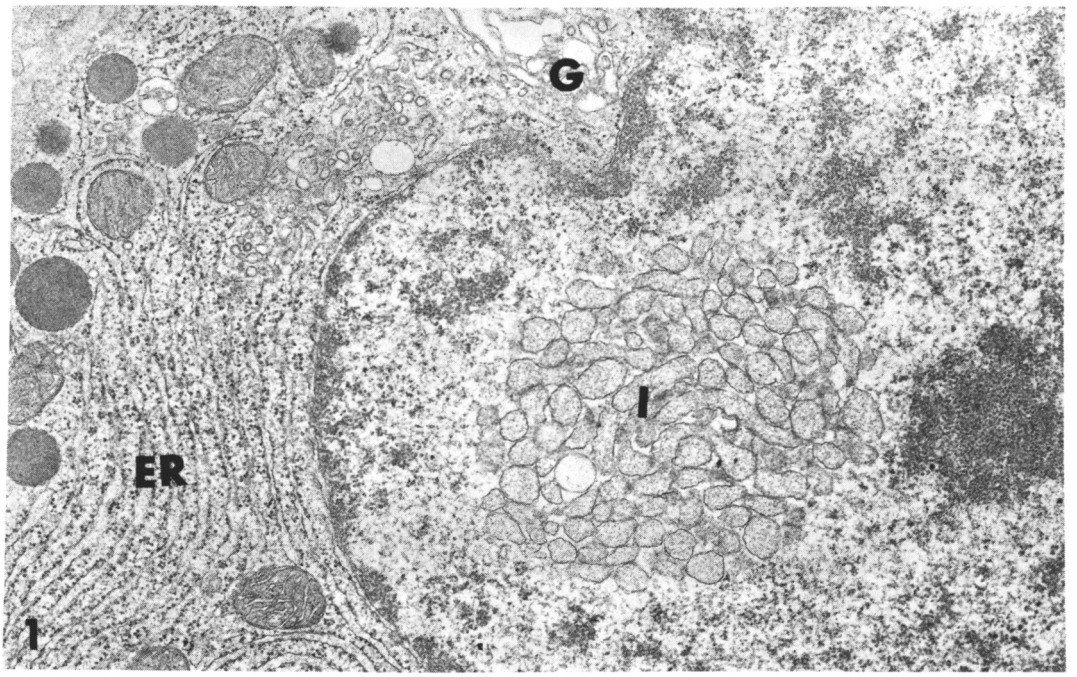

FIG. 1. Intranuclear inclusion in mammotroph from gerbil treated with nafoxidine and estradiol. The inclusion (I) is composed of membranous cisternae. Rough endoplasmic reticulum (ER) is prominent. Golgi apparatus (G). × 22,400.

tor to prevent some combination of estrogen with pituitary receptors.

Estrogen may act directly upon the mammotroph to induce the inclusions inasmuch as nafoxidine inhibits incorporation of estradiol into the pituitary gland (5). Alternatively, estrogen accumulates in nuclei of the hypothalamus (6). Mammotrophs are under the control of an inhibiting as well as releasing factor (4); the effect of estrogen or antiestrogens on hypothalamic releasing or inhibiting factors in the gerbil is not known.

It is especially interesting that the antiestrogen did not prevent the estrogen-induced hypertrophy of the rough endoplasmic reticulum or Golgi apparatus. Estrogen stimulates the synthesis and release (7) of prolactin and produces extensive proliferation of rough endoplasmic reticulum in mammotrophs (8).

Summary. Nafoxidine, an anti-estrogen, inhibited the induction of intranuclear inclusions by estradiol in mammotrophs of the male Mongolian gerbil. No inclusions were observed with inhibitor alone or in control-injected gerbils. Estrogen may well act directly on mammotrophs in induction of nuclear inclusions.

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