Pituitary Receptor Site Blockade by a Gonadotropin-Releasing Hormone Antagonist in vivo: Mechanism of Action

Abstract. *Administration of a potent gonadotropin-releasing hormone (GnRH) antagonist [Nac-L-Ala[1],pCl-D-Phe[2],D-Trp[3,6]]GnRH as a single subcutaneous injection to castrated adult male rats reduced, by more than 90 percent, both serum luteinizing hormone concentrations and specific pituitary GnRH receptor binding. This effect persisted for 24 hours. The dissociation rate of the antagonist from pituitary membrane homogenates was fourfold slower than the dissociation rate of a potent agonist. The prolonged in vivo inhibition of pituitary GnRH receptor binding and luteinizing hormone secretion by the GnRH antagonist may be mediated by the slower dissociation rate of the antagonist from its specific pituitary membrane receptor site.*

Two classes of synthetic analogs of gonadotropin-releasing hormone (GnRH) have been developed that can inhibit gonadotropin release after long-term administration. First, potent agonists of GnRH initially stimulate and subsequently inhibit gonadotropin secretion in both animal model systems and humans (*1, 2*). In the rat, short-term administration of GnRH agonists increases pituitary GnRH receptor number and stimulates gonadotropin secretion, whereas long-term administration leads to pituitary desensitization to GnRH (*3*). Potent antagonist analogs of GnRH have been developed that bind to the GnRH receptor (*4*) and inhibit rather than stimulate gonadotropin secretion (*5*). Such antagonists, by way of their inhibition of gonadotropin secretion, show antifertility effects in both male and female animals (*6, 7*).

In the present studies, the effects of a potent GnRH antagonist at two doses on gonadotropin secretion and pituitary GnRH receptor binding were determined over a 24-hour period in adult male Wistar rats. Three groups of rats that had been castrated 7 days previously received either 100 μg or 1 mg of the GnRH antagonist [Nac-L-Ala[1],pCl-D-Phe[2],D-Trp[3,6]]GnRH, which had been synthesized as described (*8*). The antagonist was administered as a single subcutaneous injection in corn oil. Control rats received vehicle alone.

At various times after injection, groups of five rats were killed and the concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the serum and of LH in the pituitary were measured by radioimmunoassays as described (*9*). Pituitary GnRH receptor binding was measured in portions of individually homogenized pituitary glands. Pituitary homogenates analyzed by similar methods yield results consistent with those obtained from pituitary membrane fractions centrifuged at 10,800*g*, except that the calculated receptor number in homogenates per milligram of protein is approximately half that found in the further purified 10,800*g* membrane pellets (*10*). In the present studies with rats given a single dose of antagonist, the percentage of specific binding of radioactive label per pituitary was analyzed directly by using Student's *t*-test in the three groups; the receptor content per milligram of protein was not calculated.

We studied the dissociation rate of the GnRH antagonist and an agonist ([D-

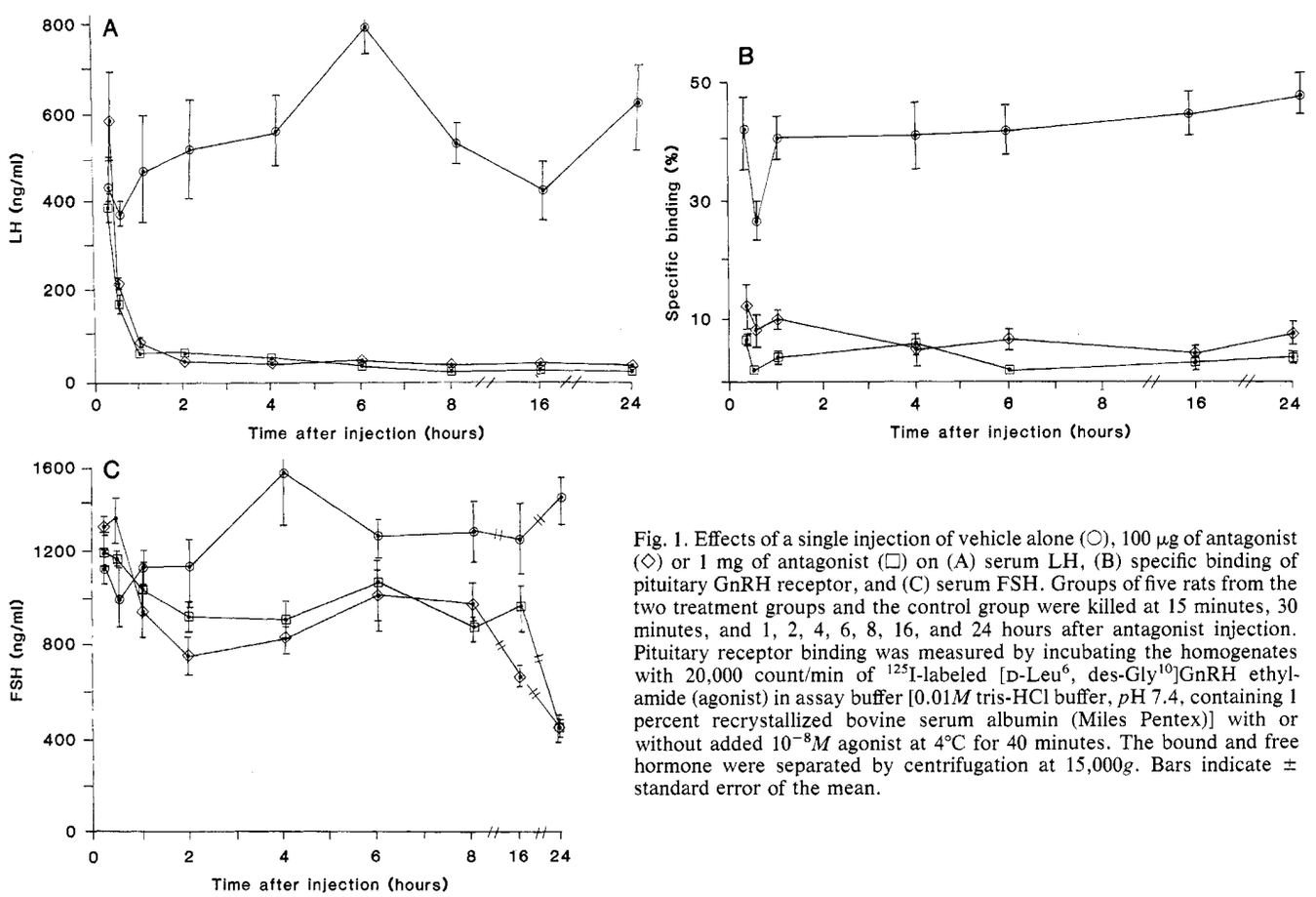

Fig. 1. Effects of a single injection of vehicle alone (○), 100 μg of antagonist (◇) or 1 mg of antagonist (□) on (A) serum LH, (B) specific binding of pituitary GnRH receptor, and (C) serum FSH. Groups of five rats from the two treatment groups and the control group were killed at 15 minutes, 30 minutes, and 1, 2, 4, 6, 8, 16, and 24 hours after antagonist injection. Pituitary receptor binding was measured by incubating the homogenates with 20,000 count/min of ^{125}I-labeled [D-Leu[6], des-Gly[10]]GnRH ethylamide (agonist) in assay buffer [0.01*M* tris-HCl buffer, *p*H 7.4, containing 1 percent recrystallized bovine serum albumin (Miles Pentex)] with or without added 10^{-8}*M* agonist at 4°C for 40 minutes. The bound and free hormone were separated by centrifugation at 15,000*g*. Bars indicate ± standard error of the mean.

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Leu^6,des-Gly^{10}]GnRH ethylamide) by labeling each with ^{125}I and purifying them as described (*11*). The labeled analogs and pituitary homogenates were incubated in the assay buffer (see legend to Fig. 1) for 40 minutes at 4°C after which either $10^{-8}M$ agonist or $10^{-8}M$ antagonist was added. After an additional 15, 30, 60, 90, or 120 minutes, bound and free hormone were separated by centrifugation at 15,000*g* and the percentage of the specific binding was determined. The percentage of added radiolabel bound was comparable for the agonist and antagonist (56 and 64 percent), whereas nonspecific binding was higher for the antagonist (14 as opposed to 5 percent). For this reason changes in the percentage of specific binding were expressed as a percentage of the control binding (prior to the addition of unlabeled antagonist) and used to calculate an approximate $t_{1/2}$ of dissociation for each iodinated compound.

Both serum LH concentration and pituitary GnRH binding decreased rapidly after injection of the GnRH antagonist (Fig. 1). The maximum decrease in serum LH occurred 1 hour after injection of either dose of antagonist. Pituitary contents of LH remained the same in the treated and control groups during the 24-hour study period.

These findings are consistent with an early inhibition of LH secretion and rapid clearance of LH from the circulation. Maximum inhibition of pituitary GnRH receptor binding by the antagonist, at both doses, occurred by 30 minutes after injection. Both serum LH and pituitary GnRH receptor binding remained suppressed for 24 hours after a single injection of antagonist at either dose. In contrast to serum LH, suppression of serum FSH (Fig. 1) was less marked and much delayed, with 50 percent suppression occurring 24 hours after injection. The responses of serum FSH to antagonist injection did not vary according to the dose used, and the decrease in FSH was delayed more than could be explained simply on the basis of the slower metabolic clearance rate of rat FSH ($t_{1/2}$ = 110 minutes) compared to LH (*12*).

When the agonist and antagonist were radioiodinated and dissociated from pituitary membranes by the addition of $10^{-8}M$ analog, the dissociation rate of the antagonist was approximately fourfold slower than that of the agonist ($t_{1/2}$ = 120 minutes compared to 30 minutes) (Fig. 2). The GnRH agonist that we used stimulates gonadotropin secretion to a maximum in rats at approximately 6 hours after administration, with a decline toward basal levels occurring by 24 hours (*13*). The duration of action of the agonist is dependent on a series of steps that includes internalization of hormone receptor complexes (*14*). Maximum inhibitory effects of the antagonist on serum LH and pituitary GnRH receptor binding occurred very rapidly compared to maximum stimulatory effects of the agonist. The slower dissociation rate of the antagonist in vitro compared to the dissociation rates of the agonist or GnRH (*15*) suggests a mechanism for the prolonged suppression of pituitary GnRH receptor binding after a single injection of antagonist.

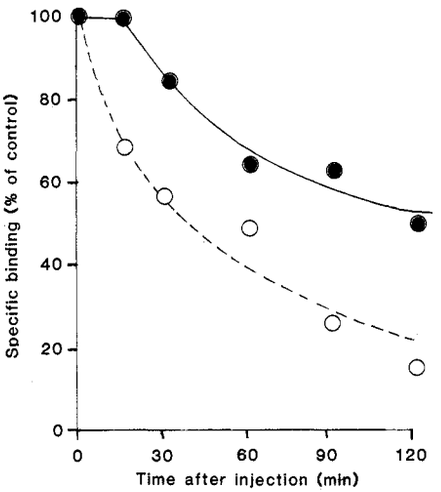

Fig. 2. Specific pituitary GnRH receptor binding (see text) for radioiodinated agonist (○) and antagonist (●) plotted against time in minutes after exposure to 10^{-8} *M* concentrations of uniodinated agonist and antagonist, respectively.

The rapid, prolonged inhibition of gonadotropin secretion caused by the GnRH antagonist is probably mediated by simple blockade of receptor binding sites. Whereas long-term treatment of intact male rats with GnRH antagonists (*4*, *16*) has been shown to lead to decreased pituitary LH and FSH, we observed no changes in pituitary LH content in rats given a single dose of antagonist, suggesting primary inhibition of the LH release mechanism.

Although the effects of long-term treatment with the antagonist on pituitary LH and FSH contents may be secondary to direct effects on LH and FSH synthesis, it is also possible that with long-term treatment the decreases in pituitary gonadotropin content are secondary to the observed decreases in gonadal steroids which might affect gonadotropin synthesis by way of hypothalamic or pituitary mechanisms. The differential sensitivity of FSH in vivo to inhibition by a single dose of the GnRH antagonist does not occur either in antagonist-treated dispersed pituitary cell cultures or in rats given long-term treatment with antagonist; such long-term treatment in vivo leads to suppression of both gonadotropins (*17*). The mechanisms for this differential sensitivity deserve further investigation.

The present studies support the view that the prolonged effects of a single GnRH antagonist injection on gonadotropin secretion are secondary to blockade of the pituitary GnRH receptor binding site. The mechanism of the prolonged blockade can be related to the slower dissociation rate of GnRH antagonist compared to agonist. The utilization of GnRH antagonists for contraception and therapy of endocrine-responsive tumors may be optimized by the design of delivery routes designed to prolong further the receptor-mediated effects of these antagonists.

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