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ANAPHYLACTOID AND ANTI-OVULATORY ACTIVITIES OF LHRH ANTAGONISTS IN RATS

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Summary

Studies were conducted with LHRH antagonists examining the relationship of structure to anaphylactoid-like activity and the relationship of anaphylactoid-like activity to anti-ovulatory activity in rats. Substitution of basic amino acids appeared to enhance the anaphylactoid-like activity of these peptides but other complex structural characteristics may also be involved. Anaphylactoid and anti-ovulatory activities were clearly independent and potent LHRH antagonists with minimal anaphylactoid-like activity were identified.

Analogs of luteinizing hormone releasing hormone (LHRH) have been proposed for use in a variety of clinical disorders that respond to an inhibition of the reproductive endocrine system such as endometriosis, precocious puberty and prostatic carcinoma. These analogs, some of which are currently being studied clinically, have included agonists as well as competitive antagonists. LHRH antagonists have a potential advantage over agonists, since they inhibit the reproductive endocrine system almost immediately and do not involve an initial stimulatory period which is required of the agonists before desensitization occurs. Considerable research efforts have been directed toward synthesizing antagonists with increased potency (1,2,3,4). The structure activity relationships of these peptides appear to be very complex and substitutions at positions 2, 3 and 6 have been the most effective in enhancing antiovulatory activity.

One of these potent LHRH antagonists [N-Ac-D-Nal(2)^1,4F-D-Phe^2, D-Trp^3, D-Arg^6]LHRH (ORF 18260) was proposed for clinical studies. We reported the reproductive endocrine properties of this antagonist previously (5,6). Toxicity studies with this peptide, however, revealed local vascular permeability changes in rodents (7) and other studies indicated that it was a potent histamine releasing agent (8). Studies in our laboratories (5,6) demonstrated that these changes were identical to cutaneous anaphylaxis reactions. We describe here studies conducted with LHRH antagonists examining the relationship of structure to anaphylactoid-like activity and the relationship of anaphylactoid-like activity to anti-ovulatory activity in rats.

Materials and Methods

Cutaneous Anaphylactoid Activity

Female Wistar-derived rats were injected intravenously with Evan's blue (1 ml

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of 0.5% solution). Evan's blue is a standard dye that, when bound to serum proteins after intravenous injection, allows one to measure a change in vascular permeability (i.e., as protein invades the interstitium it carries the dye with it and stains the connective tissue). Dilutions of peptide were injected intradermally into a shaved section on the back of the rat. Four sites were injected in each rat. Fifteen minutes after the intradermal injection, the rats were sacrificed, the dorsal skin reflected, and the area of the wheal was measured as the product of the longest perpendiculars. Regression analysis was used to estimate the dose required to produce an 8.75 x 8.75 mm wheal.

Ovulation Inhibition

Female Wistar-derived adult rats were injected subcutaneously with an LHRH antagonist dissolved in saline at 1 to 2 pm on the day of proestrus (12 hours light, 12 hours dark, lights on at 6 am). The stage of the estrous cycle was determined by examining vaginal cytology daily and only rats exhibiting at least two consecutive four-day estrous cycles were used. The presence of epithelial vaginal cells in the absence of leukocytic cells indicated proestrus. The doses tested were 0.3125, 0.625, 1.25, 2.5, 5.0 or 10.0 μg/kg. Each antagonist was tested at three or four of these doses and ten rats were tested per dose group. Control rats received saline only. The following morning, the animals were killed and the oviducts were excised and examined microscopically for the presence of ova. A rat with one or more ova present in the oviducts was considered to have ovulated. Probit analysis was used to estimate the dose required to inhibit ovulation in 50% of the rats.

Peptides

The structures of the 12 peptides tested are given in Table I. The peptides, which were kindly provided by Dr. Marvin Karten of the NIH and Dr. Jean Rivier of the Salk Institute, were formulated in saline and prepared fresh before testing.

Results

The amino acid sequences of the 12 peptides tested are presented in Table I in their order of potency in the assay measuring anaphylactoid-like activity in rats. Some trends in a structure activity relationship were seen. Peptides which did not contain arginine in position 5 or 6 tended to be less potent anaphylactoid agents. There were exceptions, however. Peptide 3, which contained D-arginine in position 6, was not a relatively potent anaphylactoid agent (ED 9.54 μg), and peptide 7, which did not contain arginine in positions 5 or 6, was moderately potent in producing a wheal response (ED 0.21 μg).

Peptide 12 (ORF 18260) which contains D-Arg^6 was the most potent anaphylactoid agent tested. This LHRH antagonist has been reported previously to be a potent histamine releasing agent (8) and to induce cutaneous vascular permeability changes in the rat (7).

Substitution of D-Arg in position 6 did not result in peptides with greater anaphylactoid-like activity than those with Arg in position 5, but rather they were equipotent. Peptide 11, which contains D-Pal(3) in position 6, exhibited relatively potent anaphylactoid activity.

The inhibition of ovulation in rats with two LHRH antagonists is shown in Fig. 1. Peptide 6 is significantly (1.7 times) more potent than peptide 12 in