tor configurations as there are calanoid species, or whether some species share the same configuration—species which do or do not inhabit the same environment—is not known.

Gravity serves two functions: (i) it gives calanoids orientational direction at night within unlimited uniform space, and (ii) it helps to set up a large feeding current. Calanoids do not possess internal gravity receptors (*26*). Their mechanoreceptors on the first antennae function, during sinking, as gravity receptors (*27*). A neutrally buoyant animal (*28*), unable to orient itself, persistently executes escape movements, uses up all its energy (*29*), and dies. During a feeding bout the animal does not sink. It uses its relative weight to create a strong feeding current and takes advantage of the double shear field to perceive its food. The extended first antennae serve, therefore, not only as parachutes (*30*) but also as sophisticated arrays of sensors, perceiving trajectories of algae, gravity, and approaching predators (*31*). A case in point is the report (*32*) that Arctic calanoids, which store large amounts of wax esters and are, therefore, positively buoyant, behave in an upside-down fashion (*33*).

In summary, negative buoyancy helps calanoid copepods to orient and to create a large laminar feeding current in which the active space around an alga is deformed predictably. Arrays of chemosensors perceive the trajectory of an alga in the feeding current. In selecting the flow through the capture area (Fig. 2A) from the feeding current, calanoid copepods maximize encounter rate with algae. This allows them to survive in nutritionally dilute environments (*1*).

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Suppression of Ovulation in the Rat by an Orally Active Antagonist of Luteinizing Hormone–Releasing Hormone

Abstract. *A synthetic antagonist of luteinizing hormone–releasing hormone blocked ovulation in rats in a dose-dependent manner when given by gavage on the afternoon of proestrus. Ovulation was delayed for at least 1 day in all animals given 2 milligrams of antagonist and in some of the animals treated with 1 or 0.5 milligram. Oral administration of 2 milligrams also blocked the preovulatory surge of luteinizing hormone. This demonstration that antagonists of luteinizing hormone–releasing hormone can have oral antiovulatory activity clearly enhances their therapeutic potential.*

The isolation, structural elucidation, and synthesis of luteinizing hormone–releasing hormone (LH-RH), which controls the secretion of luteinizing hormone (LH) and follicle-stimulating hormone from the pituitary, have opened new approaches to contraception (*1*). These approaches are based on antagonistic as well as agonistic analogs of LH-RH (*1*). Several laboratories have reported the synthesis of antagonistic analogs of LH-RH that inhibit ovulation in several species by suppressing the preovulatory surge of gonadotropins (*2*). Some of these antagonists are active in humans (*3*). However, the necessity of administering these peptides parenterally has hindered their clinical use for contraception. We now report a potent new antagonist of LH-RH that has antiovulatory effects when given orally.

The LH-RH antagonist [N-acetyl-D-*p*-chloro-Phe^{1,2},D-Trp^3,D-Arg^6,D-Ala^{10}]-LH-RH (Phe, phenylalanine; Trp, tryptophan; Arg, arginine; Ala, alanine) is synthesized by solid-phase methods and has potent antiovulatory effects in the rat (*4*). As little as 5 μg of the peptide completely inhibited ovulation in 4-day-cycling Sprague-Dawley rats when injected subcutaneously in 40 percent propylene glycol and saline at noon on the day of proestrus (*5*). All five animals injected with vehicle ovulated. We studied the analog further by determining its ability to suppress LH levels in ovariectomized rats (Fig. 1). The animals were ovariectomized 14 days before receiving

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a single injection of 10 or 100 μg of the antagonist and were decapitated at the time of injection or up to 30 hours afterward. Concentrations of LH in serum were markedly suppressed 1 and 6 hours after treatment with either dose (*6*). Twenty-four hours after treatment, serum LH rose to levels seen in the control animals.

The potency of this antagonist encouraged us to test it for oral activity also. In a second experiment we dissolved various amounts of the antagonist in 40 percent propylene glycol and saline. Trasylol, a protease inhibitor (*7*), was added (1000 I.U./ml) in an effort to retard degradation of the peptide by digestive enzymes (*8*). One milliliter of antagonist or vehicle was then given through a polyethylene tube inserted into the esophagus of conscious rats at 1400 hours on the day of proestrus. The rats had exhibited at least three consecutive 4-day cycles before treatment. The animals were deprived of food but not water from 1000 to 1530 hours on the day of the gavage.

On the morning of estrus the oviducts were examined for the presence of ova. All animals given vehicle alone ovulated; the antagonist, however, induced a dose-dependent blockade of ovulation (Table 1). Ovulation was blocked in all 11 animals given 2 mg and in 7 of 11 animals given 1 mg. Even at a dose of 500 μg, the antagonist blocked ovulation in 2 of 11 animals. In a group of six rats given 2 mg of the antagonist by gavage on proestrus, three animals had ova in their oviducts on what would have been diestrus I of the next cycle, indicating that ovulation was delayed 1 day by the antagonist. When the analog was given 1 day earlier, at 1400 hours during diestrus II, all the animals ovulated, including those that received the 2-mg dose.

The blockade of ovulation by oral administration of the analog was accompanied by suppression of the preovulatory surge of LH. The 2-mg dose suppressed the increase in LH normally seen at 1800 hours during proestrus in the control animals (Fig. 2). Since the antagonist can block ovulation when given as late as 1400 hours on proestrus, it appears to be quickly absorbed and fast-acting. This time span is consistent with the interval between oral administration of LH-RH agonists and the subsequent increase in LH (*9*).

From the minimum amount of parenterally administered antagonist needed to induce total blockade of ovulation (~ 5 μg per rat), it appears that less than 1 percent of the antagonist is absorbed when given orally. Accordingly, the amount given by gavage was relatively large. However, the finding that antagonists of LH-RH have antiovulatory effects when given orally is important. It is likely that more potent oral antagonists of LH-RH will be developed. Since known LH-RH antagonists contain so many dextrorotatory amino acids, enzymatic degradation should be severely retarded (*10*). Moreover, it may be possible to protect the antagonist further and facilitate absorption by changing the conditions of oral administration, as by using other carriers or encapsulating the peptide in liposomes (*11*).

The proven efficacy of LH-RH antagonists given orally may have far-reaching implications. In addition to serving as contraceptives, these substances may be effective in treating some hormone-dependent tumors, endometriosis, and precocious puberty by lowering the secretion of LH, follicle-stimulating hormone, and sex steroids (*1*). Recently we showed that LH-RH antagonists decrease the size of prostate tumors in rats (*12*). We observed a similar inhibition of tumor growth in rats with prostate tumors and in patients with prostate carcinoma after treatment with [D-Trp^6]-LH-RH, an agonistic analog (*13*). If the oral activity of LH-RH antagonists can be increased by further synthetic modifications of LH-RH, it may be possible to inhibit ovulation and induce tumor regression by oral peptide administration.

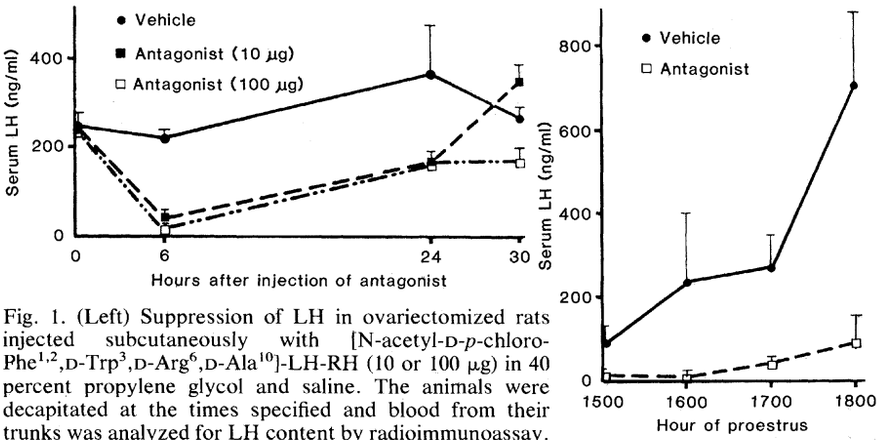

Fig. 1. (Left) Suppression of LH in ovariectomized rats injected subcutaneously with [N-acetyl-D-*p*-chloro-Phe^{1,2},D-Trp^3,D-Arg^6,D-Ala^{10}]-LH-RH (10 or 100 μg) in 40 percent propylene glycol and saline. The animals were decapitated at the times specified and blood from their trunks was analyzed for LH content by radioimmunoassay. Results are expressed in terms of NIH standard LH-RP1. Each data point represents the mean ± standard error for four determinations. Serum LH was significantly higher after 6 hours in animals injected with the vehicle than in animals given antagonist ($P < .05$). Fig. 2. (Right) Blockade of preovulatory surge of LH in proestrous rats by oral administration (gavage) of 2 mg of [N-acetyl-D-*p*-chloro-Phe^{1,2},D-Trp^3,D-Arg^6,D-Ala^{10}]-LH-RH at 1400 hours. The animals were fasted from 1000 to 1530 hours. Each data point is the mean ± standard error for four determinations. Levels of LH were significantly lower at 1800 hours in animals given the antagonist than in controls ($P < .05$), indicating that the antagonist suppressed the preovulatory surge of LH.

Table 1. Oral antiovulatory activity of [N-acetyl-D-*p*-chloro-Phe^{1,2},D-Trp^3,D-Arg^6,D-Ala^{10}]-LH-RH in rats. Different amounts of the antagonist were dissolved in 40 percent propylene glycol and 10 percent Trasylol in saline. One milliliter of antagonist or vehicle was given by gavage through a polyethylene tube inserted into the esophagus of conscious rats at 1400 hours on the day of proestrus. The data are compiled from three separate experiments.

Dose (mg/ml)	Number of animals ovulating	Number of ova per ovulating rat (mean ± standard error)
0	7 of 7	12.1 ± 0.59
0.5	9 of 11	12.3 ± 0.47
1.0	4 of 11	10.0 ± 1.41
2.0	0 of 11	

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Complete Amino Acid Sequence of Urotensin I, a Hypotensive and Corticotropin-Releasing Neuropeptide from *Catostomus*

Abstract. *Urotensin I, purified from extracts of the urophysis of a teleost fish (*Catostomus commersoni*), exhibits potent hypotensive activity (mammals and birds) and corticotropin-releasing activity (both fish and mammals). The primary structure of this 41-residue peptide was determined to be H-Asn-Asp-Asp-Pro-Pro-Ile-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Asn-Met-Ile-Glu-Met-Ala-Arg-Ile-Glu-Asn-Glu-Arg-Glu-Gln-Ala-Gly-Leu-Asn-Arg-Lys-Tyr-Leu-Asp-Glu-Val-NH_2. Extraction with 0.1*N *HCl at 100°C cleaves the amino-terminal tripeptide, yielding a fully active analog, urotensin I(4–41). The amino acid sequence was confirmed by measuring the biological activity of synthetic urotensin I(4–41). Urotensin I exhibits a striking sequence homology with ovine corticotropin-releasing factor and with frog sauvagine. These three peptides exhibit similar activities in biological test systems.*

The name urotensin and the definitions of the activities of urotensin I to urotensin IV were proposed (*1*) after the purification of a number of fish urophyseal peptides that have potent effects on smooth muscle (*2*). Urotensin I (U_I), a neuropeptide isolated from the urophysis of the teleost *Catostomus commersoni* (white sucker), has mild vasopressor activity in fish and other cold-blooded vertebrates. In contrast, the action of U_I in mammals is of particular interest because of its uniquely selective vasodilatory, hypotensive activity (*3*). During the course of our work on the structure of U_I, Vale *et al.* (*4*) described the amino acid sequence and biological properties of ovine corticotropin-releasing factor (CRF). It then became apparent to us, even at a preliminary stage of our sequence studies, that U_I is structurally similar to ovine CRF and to the related frog skin peptide, sauvagine (*5*). We have now determined the complete amino acid sequence of intact U_I (a 41-residue peptide) and of a fully active analog, U_I(4–41), both of which can be obtained from acid extracts of *C. commersoni* urophyses under appropriate conditions. Further, we have compared the biological activities of naturally occurring U_I with the activities of synthetic U_I, ovine CRF, and sauvagine.

Urotensin I was extracted in 0.1*N* HCl from acetone-dried *C. commersoni* urophyses, either with or without a heating step. Heating at 100°C for 15 minutes (*6*) led to the extraction of active peptide material, which was readily purified by the chromatographic procedures but proved to be a partially hydrolyzed product [U_I(4–41); see below] of the intact peptide present in unheated acid extracts. Because of the comparatively higher yield of peptide from the heated extracts, heat treatment was used for the preparation of peptide used for most of the sequence studies.

Throughout the purification, U_I activity was monitored by measuring the decrease in perfusion pressure in the isolated rat hind limb assay (*7*); the U_I activity was expressed in units defined earlier (*1*). Polyacrylamide gel electrophoresis (PAGE) (7.5 percent acrylamide; tris-HCl, *p*H 9.5; staining by Coomassie blue R; trichloroacetic acid) was also used as a guide for pooling active fractions (*8*).

Peptides were isolated from batches (500 mg) of acetone-dried urophyses (~ 2000 urophyses per batch) and purified by sequential gel filtration, ion-exchange chromatography, and reversed-phase high-performance liquid chromatography (HPLC) (*9*). The initial 0.1*N* HCl extracts contained 12 to 23 units of U_I activity per milligram of protein. Protein content in extracts and fractions was determined with ninhydrin after alkaline hydrolysis or by amino acid analysis, respectively.

Urotensin I was isolated as outlined in the legend of Table 1. Amino acid analyses, after SP-Sephadex chromatography or HPLC, showed close to integral molar ratios for both peptides (Table 1) and, together with sodium dodecyl sulfate–PAGE (*10*), suggested 38 residues for the peptide extracted with the heating step and 41 residues for the peptide extracted without the heating step.

Edman degradation of U_I(4–41) (*11*, *12*) and of fragment mixtures derived from trypsin, *Staphylococcus aureus* protease (SAP), and cyanogen bromide (CNBr) cleavage (*11*), provided a sequence identification for residues 4 to 38 (Fig. 1). Carboxypeptidases A, B, or Y did not release amino acids from the carboxyl terminus of U_I(4–41), suggesting a blocked or conformationally restricted carboxyl terminus. Tryptic, SAP, and CNBr fragments were therefore isolated by HPLC (conditions as given in Table 1 but with various acetonitrile gradients) and characterized by amino acid analysis; the resultant fragments were used to complete the sequence (*13*). Tryptic digests subjected to HPLC yielded two tyrosine-containing peptides, a pentapeptide and a hexapeptide containing an additional lysine. Edman degradation of the hexapeptide gave the sequence Lys-Tyr-Leu-Asp-Glu- (*14*). Only valine remained unaccounted for from the amino acid analysis on the hexapeptide. Valine amide was liberated (35 percent yield after HPLC) by SAP digestion of U_I(4–41) in 0.05*M* ammonium acetate, *p*H 4.0, and identified (*15*) by (i) thin-layer chromatography under conditions that separate valine and valine amide [silica-gel 60 F-254 (Merck); butanol, pyridine, acetic acid, and water (15:10:3:6); and ninhydrin] and (ii) hydrolysis (6*N* HCl for 20 hours at reduced pressure) to valine, identified by amino acid analysis (there was no valine peak in

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