these potentials are generated at the same site or at different sites (for example, at the initial segment or dendrites) is still unknown.

To our knowledge this represents the first report in which intracellular recordings from neurons in the mammalian central nervous system have been obtained simultaneously with evidence for their neurochemical identity. It also represents the first step in the intracellular characterization of brain monoamine neurons recorded in vivo. These techniques should make possible investigations in much greater depth of various aspects of this neuronal system, for example, the function of autoreceptors (*9*, *18*), depolarization inactivation (*19*), burst firing (*7*), effects of afferent inputs on dopaminergic cell membrane properties, and the mechanisms of action of dopamine agonists and antagonists. Data obtained from such studies may further our understanding of dopamine system function in both normal and pathological states.

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11. Glass micropipettes were filled with 3*M* potassium acetate (for electrophysiological recordings) or 1*M* L-dopa methylester in 1*M* lithium chloride (for L-dopa injection) and beveled to an impedance of 35 megohms measured at 1000 Hz with a modification of the method of T. E. Ogden, M. C. Citron, and R. Pierantoni [*Science* **201**, 469 (1978)].
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Bioactive Conformation of Luteinizing Hormone-Releasing Hormone: Evidence from a Conformationally Constrained Analog

Abstract. *An analog of luteinizing hormone-releasing hormone containing a γ-lactam as a conformational constraint has been prepared with the use of a novel cyclization of a methionine sulfonium salt. The analog is more active as a luteinizing hormone-releasing hormone agonist than the parent hormone, and provides evidence for a bioactive conformation containing a β-turn.*

Peptide structures normally exist in solution as an equilibrium mixture of conformers. Backbone conformational constraints are of interest as a means of limiting the number of conformations available to the peptide (*1*). Potential advantages to be realized with the use of these restrictions in biologically active peptides include increasing the potency by stabilizing a biologically active conformer (*2*), decreasing degradation by eliminating metabolized conformers, and improving biological selectivity through elimination of bioactive conformers that give undesired biological responses (*3*). In addition, information can be obtained about the biologically active conformation of the peptide at a specific receptor through the introduction of the conformational constraint (*4*).

Two basic types of conformational modifications have been used in analogs of biologically active peptides which place limits on the possible bioactive conformations. Noncovalent modifications include D amino acids (*5*), *N*-methyl amino acids (*6*), and α-methyl amino acids (*1*). Most frequently applied and successful among these have been the D amino acids. Covalent modifications forming cyclic and polycyclic peptides include as more common examples cyclic amino acids such as proline (*7*) and disulfide bridges (*8*) and cyclization through amide bonds (*7*), all of which are known to occur in nature. β-Lactams appear as naturally occurring modifications in the penicillins and cephalosporins and serve also as reactive agents (*9*). We have been exploring the use of larger ring (five-, six-, and seven-membered) lactams as novel conformational modifications in peptides (*10*) and report here a lactam-containing analog of luteinizing hormone-releasing hormone (LH-RH) more active than the parent hormone.

Since the determination of the sequence of LH-RH (<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) (*11*), many analogs have been prepared (*12*). Because of its higher potency, one of the useful structural modifications is the substitution of a D amino acid for the glycine residue in position 6 (*13*). For example, the D-Ala^6 analog is 3.7 times as active as LH-RH itself. In contrast, the L-Ala^6 analog has low potency. A second noncovalent constraint resulting from replacement of Leu^7 by *N*-methyl-Leu gave a further increase in activity (*14*). The enhanced biological activity of the D-Ala^6-*N*-methyl-Leu^7 analog is consistent with a β-turn conformation for residues 5 to 8 of LH-RH (*5*). An amino acid of the L configuration in position 6 should destabilize this β-turn, which is the presumed reason for the low activity of such analogs. The presence of a β-turn is predicted also by conformational energy calculations (*15*). The theoretical calculations also predict a stabilization of this conformation in D-Ala^6 analogs and a destabilization in L-Ala^6 analogs (*16*).

The proposed β-turn for LH-RH (*15*) is shown in Fig. 1, **a**. This case seemed ideally suited to the use of a lactam conformational constraint because of the proximity of the pro-*S* hydrogen atom of Gly^6 to the N^{α}-hydrogen of Leu^7. By replacing these two hydrogens with methylene groups and connecting the newly introduced carbon atoms with a

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single bond, a five-membered lactam is created (Fig. 1, **b**). Computer superposition (*17*) of these two structures (Fig. 1) shows good correspondence for the backbones of the two peptide fragments. Indeed, the average deviation of six pairs of matched atoms along the backbones is 0.13 Å. The new ring should stabilize the β-turn conformation by restricting rotation about the dihedral angle ψ_6 (*18*) and forcing the Gly-Leu peptide bond to remain *trans* (ω_6). The lactam also affects ϕ_6 and ϕ_7 as a noncovalent constraint. A key point about this lactam is that the 6-position α-carbon in the ring must have the L configuration to fit the proposed β-turn. Since L amino acids at position 6 reduce biological potency, this case is an excellent test for the utility of the lactam constraint as a conformational probe.

The synthesis of the required compound **1** (Fig. 2) incorporates a novel preparation of the γ-lactam as the key step. The protected dipeptide **2** was converted to its sulfonium salt **3** in methyl iodide. Stereospecific cyclization to the lactam **4** (*19*) was achieved in 50 percent yield by treatment with sodium hydride in a mixture of methylene chloride and dimethylformamide (1:1) (*20*). This is the first observation of an intramolecular *N*-alkylation of a methionine sulfonium salt. Normally the cyclization of methionine sulfonium intermediates results in amide *O*-alkylation and is used extensively for peptide degradation and sequence studies (*21*). Generation of the amide anion with strong base changes the course of reaction in this case. A *C*-alkylation of methionine sulfonium salts has also been reported (*22*). The key synthetic step allows formation of the basic ring system with the definition of chirality at two centers introduced by the use of the readily available optically pure amino acids (*22a*). The γ-lactam **4** was coupled with tripeptide **5** with the use of diphenylphosphoryl azide (*23*) to produce the pentapeptide **6** in 80 percent yield. This fragment was deblocked and coupled to pentapeptide **7** (*24*) by the azide method to give the conformationally constrained LH-RH analog **1** (*25*).

Compound **1** shows greater potency than LH-RH for inducing release of LH when tested both in vivo and in vitro. The ability of **1** to cause LH release in vivo was evaluated in adult ovariectomized female rats (Holtzman) primed with estradiol and progesterone (*26*). The analog was given intravenously in graded doses 20 minutes before the animals were bled, and LH levels in plasma were determined by radioimmunoassay (*27*). Comparison with similar doses of LH-RH showed **1** to be 2.4 [95 percent confidence limit (CL); 0.8, 7.6] times as potent.

The potency of **1** was also evaluated relative to LH-RH in an in vitro pituitary cell culture system (*28*). Dispersed cells were incubated with LH-RH or **1** at various concentrations (10^{-6} to $10^{-10}M$) for 1 hour and the LH content of the supernatant was determined by radioimmunoassay. Compound **1** was found to be 8.9 (95 percent CL; 2.0, 38.6) times as potent as LH-RH. The high potency in this in vitro system where the influence of metabolism is minimized suggests that the increased potency is a result of improved

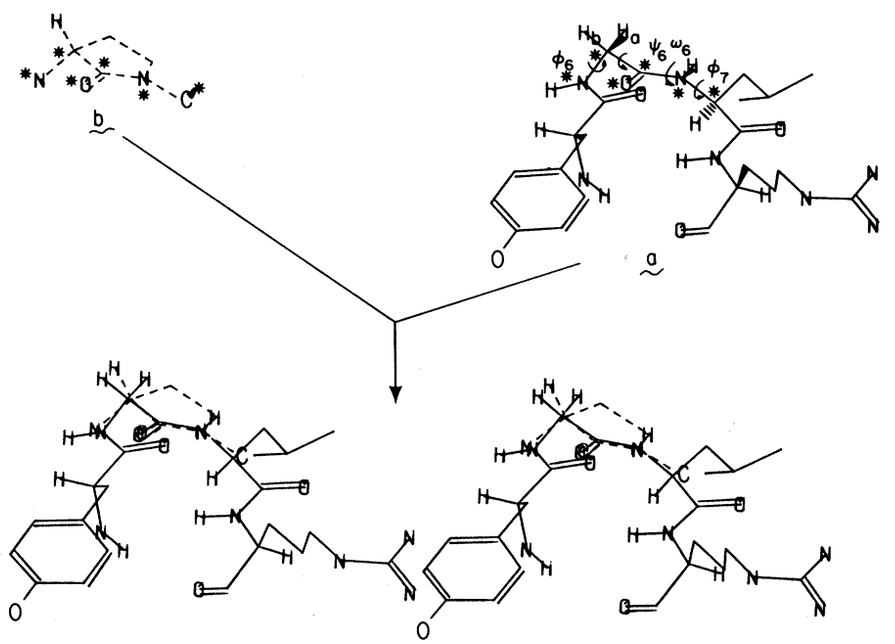

Fig. 1. Computer superposition of β-turn segment of LH-RH (Tyr-Gly-Leu-Arg) (**a**) with γ-lactam conformational constraint (**b**). Average deviation of least squares fit of matched atoms (starred) is 0.13 Å. H_a and H_b are the pro-*S* and the pro-*R* hydrogen atoms of glycine, respectively. Dihedral angles affected by the constraint are shown by curved arrows.

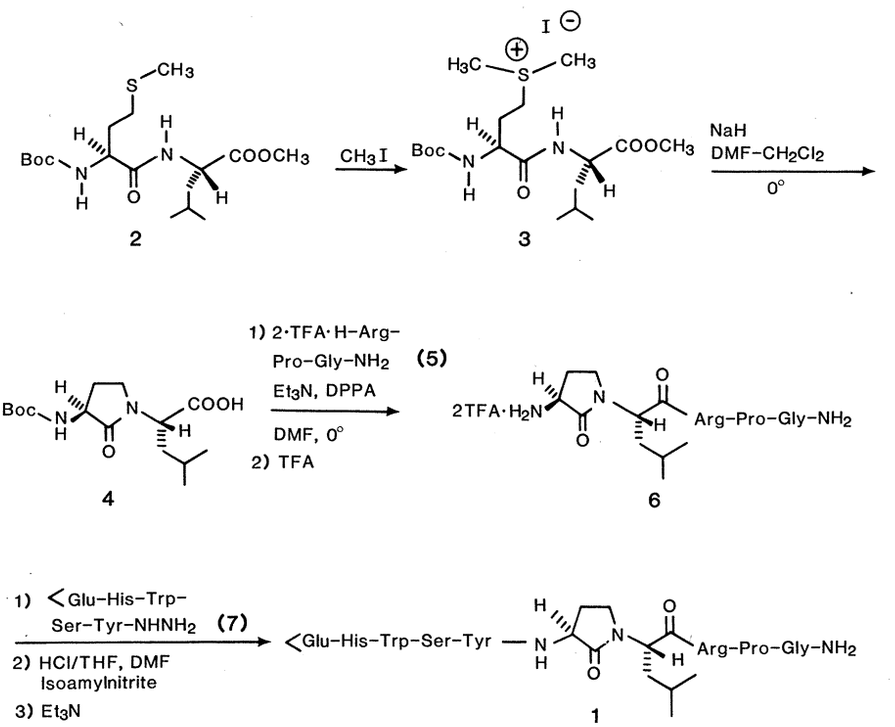

Fig. 2. Synthesis of conformationally constrained LH-RH analog **1**. Abbreviations: *Et*, ethyl; *Boc*, *tert*-butyloxycarbonyl; *DMF*, dimethylformamide; *TFA*, trifluoroacetic acid; *DPPA*, diphenylphosphoryl azide; *THF*, tetrahydrofuran.

receptor binding and not of increased resistence to proteases.

The biological data obtained for analog **1** provide additional support for a receptor-bound conformation of LH-RH which contains a Tyr-Gly-Leu-Arg type II′ β-turn. Other types of β-turns known to exist in proteins (*29*) have also been examined by computer superposition with the lactam peptide. All of these accommodate the lactam ring less well. Conformations are possible that can accept the lactam and do not contain a turn. However, the enhanced potencies obtained with three different conformational constraints (D amino acids, *N*-methyl amino acids, and lactams), all of which would stabilize a turn structure, provide strong evidence for the existence of this type of structure in LH-RH when bound to the receptor in such a way as to produce a biological response. The results also indicate that the loss of activity with the L-alanine substitution in position 6 was due to destabilization of the favored conformation rather than some steric interaction with the receptor.

This successful demonstration of the application of a lactam as a new type of conformational constraint in peptides providing inference of bioactive conformation and increased biological potency suggests future applications. With the newly developed synthetic methodology, a variety of five-membered lactam-containing dipeptides can be synthesized for incorporation into specific peptides. We have previously shown the stabilization of a γ-turn structure by a six-membered lactam (*10*). These structures can complement currently used conformational constraints by adding to the information obtainable from a conformation-activity approach, thereby facilitating the design of peptide analogs of improved biological activity and duration of action.

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Infectious Diseases and Population Cycles of Forest Insects

Abstract. *The regulation of natural populations of invertebrate hosts by viral, bacterial, protozoan, or helminth infections is discussed, using models that combine elements of conventional epidemiology (where the host population is assumed constant) with dynamic elements drawn from predator-prey studies; the apparent absence of acquired immunity in invertebrates simplifies the analysis. Highly pathogenic infections, with long-lived infective stages, tend to produce cyclic behavior in their host populations. The models give an explanation of the 9- to 10-year population cycles of the larch bud moth (*Zeiraphera diniana*) in the European Alps and suggest that microsporidian protozoan and baculovirus infections may be responsible for the 5- to 12-year population cycles observed in many temperate forest insects.*

It is possible that parasitic infections—broadly defined to include viruses, bacteria, protozoans, and helminths—may regulate the population density of their hosts (*1*). Recent research (*2*) combines theoretical models with field and laboratory data, seeking to fuse two well-established but separate literatures: classical epidemiology, which treats the maintenance and transmission of infections within a host population that has a constant magnitude, determined by other factors (*3, 4*), and predator-prey studies, which occupy a chapter in any current ecology text and deal with the way prey populations may be regulated by the predators that eat them (*5*).

This report deals in particular with the regulation of populations of invertebrate species by infectious diseases. Such systems are of special interest for at least two reasons. (i) They have important practical applications in pest control. (ii) The dynamics are somewhat simpler, and the relevant parameters more amenable to measurement, than is the case for most vertebrate host-parasite systems, because it appears that invertebrates do not develop acquired immunity to the agents of infectious disease (*6*).

In the simplest case, we define $X(t)$ to be the number of susceptible hosts and $Y(t)$ the number of infected hosts at time t; the total population of invertebrate hosts is thus $N(t) = X(t) + Y(t)$. We further define a to be the per capita birth rate of the hosts, b their natural death rate, α the disease-induced death rate of infected hosts, and γ the recovery rate. These are all quantities that may, in principle, be measured directly. In this simplest model for a directly transmitted infection (*7*), the transmission rate is assumed proportional to the number of