translocation of the molecule, and the larger the radius from which such counterproductive paths can be selected. Net translocation of the molecule must require constant extrication of trailing segments from paths that are favored locally, but which are not sufficiently favorable to lead to rerouting of the entire molecule. This heuristic view is compatible with previous data indicating that DNA molecules adopt a high-mobility conformation during steady-state electrophoresis and that relaxation from this conformation is both slow and size-dependent (*10*).

With respect to the bearing of these observations on transverse-field experiments, it is notable that all published transverse-field geometries have involved variable, but generally obtuse, angles between the applied fields (*4–6*). Indeed, we were led to attempt FIGE experiments after having observed that acute angles between transverse fields are ineffective while obtuse angles give rise to strong size-dependence. It is likely that much of the fractionation observed in transverse-field alternation experiments arises at wide angles of intersection between the transverse fields and is associated with the same head-to-tail conformational change that is the basis of the FIGE technique.

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9. For example, the 15- to 50-kb window can be opened even more dramatically than in Fig. 1B by employing applied voltages of 350 V forward/250 V reverse with a constant switching interval of 0.3 seconds for 18 hours.
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12. The computer control was achieved by using the parallel port on the IBM PC's Monochrome Display and Printer Adapter. The pins of the adapter's 25-pin D-shell connector provide a readily programmed source of standard transistor-transistor logic (TTL) output. Pins 2 to 9 are at +5 V relative to ground (pins 18 to 25) when bits 0 to 7, respectively, are set at I/O address hex 3BC, and they are at ground when the corresponding bits are not set. For example, the BASIC statement OUT &H3BC,&HA places pins 3 and 5 at +5 V and the remaining pins at ground. Since BASIC allows access to the PC's clock via the TIMER function (Versions 2.0 and later releases), it is straightforward to program switching-interval ramps. Each programmed pin can control a separate 110-V circuit by connecting the programmed pin and a ground pin to the TTL side of a Sigma (Braintree, MA) Series 226 model 226R1-5A1 Solid-State Relay; the line-voltage side of the relay will then be closed when the programmed pin is at +5 V and open when it is at ground.

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Stimulation of Gonadotropin Release by a Non–GnRH Peptide Sequence of the GnRH Precursor

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The human gonadotropin-releasing hormone (GnRH) precursor comprises the GnRH sequence followed by an extension of 59 amino acids. Basic amino acid residues in the carboxyl terminal extension may represent sites of processing to biologically active peptides. A synthetic peptide comprising the first 13 amino acids (H · Asp-Ala-Glu-Asn-Leu-Ile-Asp-Ser-Phe-Gln-Glu-Ile-Val · OH) of the 59–amino acid peptide was found to stimulate the release of gonadotropic hormones from human and baboon anterior pituitary cells in culture. The peptide did not affect thyrotropin or prolactin secretion. A GnRH antagonist did not inhibit gonadotropin stimulation by the peptide, and the peptide did not compete with GnRH for GnRH pituitary receptors, indicating that the action of the peptide is independent of the GnRH receptor. The GnRH precursor contains two distinct peptide sequences capable of stimulating gonadotropin release from human and baboon pituitary cells.

GONADOTROPIN-RELEASING HORmone (GnRH) is a decapeptide, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly · NH_2, that stimulates release of the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). GnRH and its analogs also affect the central nervous system, gonads, and placenta, and GnRH-binding sites and GnRH-like peptides have been detected in these tissues (*1*).

Although in early reports an enzyme-template mechanism was suggested to underlie the biosynthesis of GnRH (*2*), the identification of higher molecular weight immunoreactive GnRH in sheep and pig hypothalamus (*3*) and human placenta (*4*) implicated conventional ribosomal biosynthesis of a prohormonal form. This conclusion was supported by cell-free translation of hypothalamic messenger RNA (mRNA) into peptides immunoprecipitable by GnRH antiserum (*5*). Our proposal that the pyroglutamic acid (pGlu) residue at the amino terminus of GnRH is derived from glutamine while the Gly · NH_2 of the carboxyl-terminus results from enzymic processing of glycine followed by a pair of basic amino acids in the GnRH precursor (*3*) was recently definitively demonstrated by the elucidation of the nucleotide sequence of a human placental GnRH mRNA (*6*). The GnRH sequence is followed by a cleavage and amidation site (Gly-Lys-Arg) and then a 53–amino acid sequence and a second potential cleavage site (Lys-Lys-Ile) (Fig. 1). In addition to cleavages at these pairs of basic amino acid residues, processing may occur at single basic amino acids (Fig. 1), as such cleavages occur in several peptide precursors (*7*).

Posttranslational processing of several peptide precursors gives rise to different peptide fragments with biological functions

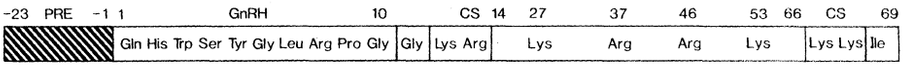

Fig. 1. Schematic diagram of human placental pre-pro-GnRH showing the signal sequence (PRE), the GnRH sequence, the carboxyl terminal extension, and putative cleavage sites (CS) at pairs of basic amino acids. Single basic amino acid residues, which may be additional sites of cleavage, are also indicated.

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(*7*). It was of interest, therefore, to determine whether this also pertained to the GnRH precursor. We synthesized some of the peptide sequences that reside between basic amino acid residues and found that a peptide comprising the first 13 amino acids of the carboxyl terminal extension peptide specifically stimulates LH and FSH release from human and baboon pituitary cells in culture. At the time this report was submitted, Nikolics *et al.* (*8*) reported that the entire carboxyl terminal extension peptide (sequence 14 to 69) exhibits both gonadotropin-releasing and prolactin-inhibiting properties in cultured rat pituitary cells.

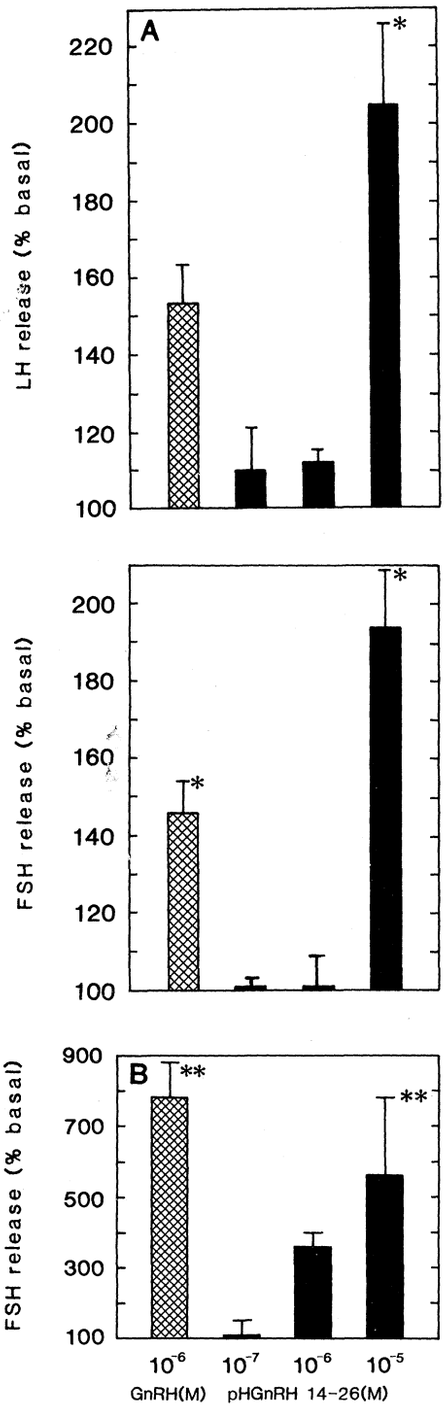

Fig. 2. Effect of GnRH and pHGnRH(14–26) on LH and FSH release from cultured human (A) and baboon (B) anterior pituitary cells compared with cells incubated with medium alone (basal). Values are means ± SEM (A: $n = 3$; B: $n = 4$). Asterisks indicate significant differences from basal values: (*) $P < 0.005$, and (**) $P = 0.05$. For baboon pituitary cell cultures, only FSH was measured, as baboon LH does not cross-react in the human LH radioimmunoassay.

The peptide H · Asp-Ala-Glu-Asn-Leu-Ile-Asp-Ser-Phe-Gln-Glu-Ile-Val · OH was synthesized by solid-phase methodology (*9*); it is designated pHGnRH(14–26), representing the precursor (p) of human (H) GnRH and the amino acid numbering assigned by Seeburg and Adelman (*6*) from the mRNA sequence (Fig. 1). Human and baboon anterior pituitary cells were cultured and incubated with GnRH and pHGnRH(14–26) (*10*).

Both GnRH and pHGnRH(14–26) stimulated secretion of gonadotropins from the cultured human and baboon pituitary cells (Fig. 2). In five separate experiments with different cultures of human pituitary cells, pHGnRH(14–26) significantly ($P < 0.005$) stimulated secretion of LH (126 to 360 percent) and FSH (159 to 831 percent). The variation in stimulation is due to differences in responsiveness of individual cultures, which were prepared from different pituitary glands for each experiment.

The effect of pHGnRH(14–26) on gonadotropin secretion was specific, since secretion did not occur in the absence of extracellular calcium, and secretion of prolactin and thyrotropin into the medium was unaffected by the peptides (*11*). Although pHGnRH(14–26) did not significantly affect prolactin secretion from human pituitary cells, the longer sequence (14–69) was recently shown to inhibit prolactin secretion from rat pituitary cells in culture (*8*). Stimulation of gonadotropin release by pHGnRH(14–26) appeared to be species-specific, as the peptide did not significantly affect LH secretion from sheep pituitary cells prepared and cultured under identical conditions but did significantly stimulate FSH release from baboon pituitary cells (Fig. 2). This contrasts with the universal activity of the known hypothalamic releasing hormones in a wide range of mammalian species.

The cultured human pituitary cells were much less sensitive to GnRH stimulation than rat (*8*) or sheep (*12*) pituitary cells, as higher doses of GnRH were required to elicit significant stimulation of gonadotropin release. However, human pituitary membrane receptors had a relatively high affinity for GnRH—similar to that of the rat receptors (Table 1). The thyrotropin response to thyrotropin-releasing hormone in human pituitary cells was less sensitive than that in the rat. The low sensitivity of human gonadotrophs to GnRH might be a consequence of changes in the GnRH receptors or postreceptor mediating mechanisms during culturing. The cultured human gonadotrophs were less sensitive to pHGnRH(14–26) than to GnRH in their LH and FSH responses; pHGnRH(14–26) may be less active because the synthetic peptide is not identical to the naturally processed carboxyl terminus peptides. Rat pituitary cells in culture were more responsive to the entire carboxyl terminal peptide pHGnRH(14–69) (*8*). Whether these are species-specific differences or are due to intrinsic activities of the 14–69 and 14–26 peptides is not known.

In view of the structural dissimilarity between pHGnRH(14–26) and GnRH, it is unlikely that pHGnRH(14–26) stimulation of gonadotropin is mediated by binding to the GnRH receptor. We therefore studied the effects of a GnRH antagonist on pHGnRH(14–26) stimulation of gonadotropin and the ability of pHGnRH(14–26) to bind GnRH receptors. Addition of a GnRH antagonist to the cultured human pituitary cells did not significantly inhibit the LH and FSH responses to pHGnRH(14–26) (Fig. 3). The antagonist was effective in blocking LH and FSH responses to GnRH at this dose, indicating that pHGnRH(14–26) does not significantly stimulate the gonadotrophs through the GnRH receptor. This conclusion is supported by the inability of pHGnRH(14–26) to compete with ^{125}I-labeled GnRH analog for binding to human anterior pituitary cell membranes (Table 1). Moreover, when added together, GnRH and pHGnRH(14–26)

Table 1. Effect of pHGnRH(14–26) on ^{125}I-labeled GnRH agonist binding to human pituitary membranes. Human pituitaries were obtained from two women 20 to 30 years of age within 12 hours of their accidental deaths. Pituitary membranes were prepared and binding studies conducted (*15*). The apparent dissociation constants of GnRH for the human and rat pituitary receptors were 4.8 ± 1.5 and 4.7 ± 1.1 n*M*, respectively.

Peptide concentration	Percentage of total binding
	100.0 ± 3.0
GnRH agonist	
$10^{-10}M$	79.1 ± 2.8
$10^{-9}M$	35.1 ± 2.8
pHGnRH(14–26)	
$10^{-6}M$	108.1 ± 14.9
$10^{-5}M$	92.9 ± 20.6

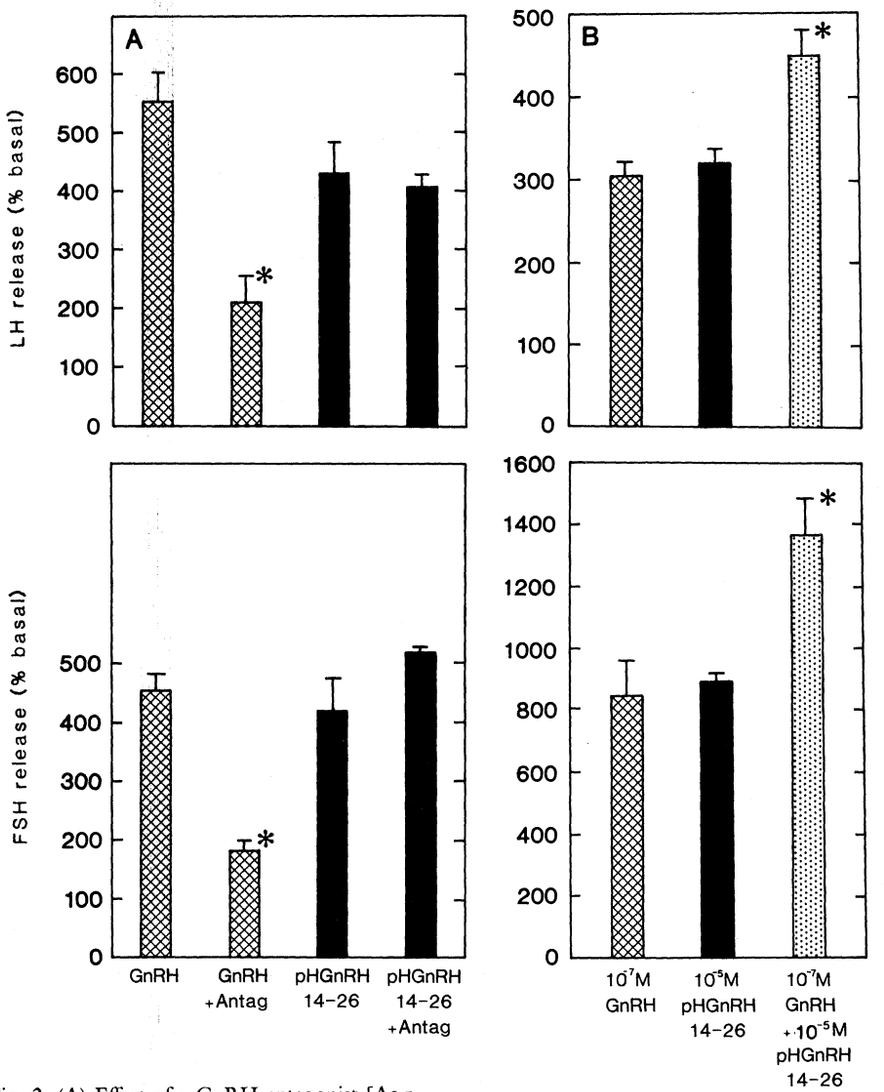

Fig. 3. (A) Effect of a GnRH antagonist [Ac-D-Nal(2)^1,D-α-Me-4-Cl-Phe^2,D-Trp^3,D-Arg^6,D-Ala^{10}-GnRH] on GnRH- and pHGnRH(14–26)-stimulated LH and FSH release. The cultured pituitary cells were stimulated with $10^{-6}M$ GnRH or $10^{-5}M$ pHGnRH(14–26) in the presence or absence of $10^{-6}M$ GnRH antagonist. (B) Effect of GnRH and pHGnRH(14–26) on LH and FSH release when added alone or in combination. Values are means ± SEM (n = 3). Asterisks indicate significant differences from values for GnRH- or pHGnRH(14–26)-stimulated release.

induced a significantly greater release of LH and FSH than either one alone (Fig. 3B).

The occurrence of two separate structurally unrelated peptides in the GnRH precursor that stimulate gonadotropin release appears to be unique among prohormones. A second stimulator of gonadotropin secretion is perhaps unexpected in view of studies demonstrating a predominant role for GnRH in this function. Administration of GnRH antiserum or GnRH antagonists to laboratory animals, or immunization with GnRH conjugates, results in a decline of circulating LH and FSH (*13*). These treatments do not, however, reduce gonadotropin to undetectable levels, and there is evidence of other hypothalamic regulators of gonadotropin (*14*).

Whether pHGnRH(14–26) or longer sequences have a physiological role in gonadotropin secretion remains to be determined.

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9. The fully protected pHGnRH(14–26) peptide was synthesized in a stepwise manner on chloromethyl resin. *t*-Butyloxycarbonyl amino acid couplings were repeated until no ninhydrin color remained (sometimes in the presence of 1-hydroxybenzotriazole and a half-equivalent of triethylamine), and all difficult couplings were followed by acetylation. Cleavage from the resin and concomitant deprotection were achieved with redistilled hydrogen fluoride, and the crude peptide was extracted from the resin with aqueous ammonia. The peptide was purified to greater than 90 percent by preparative reversed-phase high-performance liquid chromatography with a 0.1*N* ammonium acetate (*p*H 6.5) and acetonitrile buffer system. The purified peptide was characterized by sequence analysis (performed by W. Brandt) on a gas-phase sequencer.
10. Human anterior pituitaries were obtained at autopsy from human males 16 to 40 years of age 11 to 18 hours after traumatic accidental death; baboon anterior pituitaries were obtained within 30 minutes of death of the animals. These tissues were processed for cell culture (*12*), and cells were placed in culture dishes (35 by 13 mm, Falcon 3046), with each dish containing 2×10^5 to 6×10^5 cells in 2 to 3 ml of minimum essential medium (MEM) with Hanks' salt solution supplemented with $NaHCO_3$ and 10 percent donor calf serum. The cells were cultured for 2 to 3 days in a humidified incubator at 37°C with 95 percent O_2 and 5 percent CO_2. Viability of dispersed cells, as assessed with trypan blue, was 50 to 80 percent. Nonviable cells failed to attach to culture wells. After 2 to 3 days in culture the pituitary cells were washed six times with serum-free medium, and the test peptides were added to wells in duplicate or triplicate. After 3 hours of incubation the medium was removed and assayed for LH, FSH, prolactin (PRL), and thyrotropin-stimulating hormone (TSH) immunoreactivity. Assays were performed in duplicate with a human LH radioimmunoassay (Amerlex LH RIA kit IM 2081, Amersham), a human FSH radioimmunoassay (Amerlex FSH RIA kit IM 2071, Amersham), a human TSH radioimmunoassay (Amerlex TSH RIA kit IM 3161, Amersham), and a human PRL radioimmunoassay (PRL RIA kit 10803, Serono Diagnostics).
11. Prolactin concentrations were: control, 7.24 ± 0.07 ng/ml; $10^{-6}M$ GnRH, 7.54 ± 0.72 ng/ml; and $10^{-5}M$ pHGnRH(14–26), 8.70 ± 0.60 ng/ml. Thyrotropin concentrations were: control, 1.51 ± 0.20 μU/ml; $10^{-6}M$ GnRH, 1.26 ± 0.77 μU/ml; and $10^{-5}M$ pHGnRH(14–26), 1.86 ± 0.35 μU/ml.
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15. The preparation of human pituitary membranes and the GnRH receptor binding assay have been described (P. J. Wormald, K. A. Eidne, R. P. Millar, *J. Clin. Endocrinol. Metab.*, in press). Unlabeled peptides (50 μl) and 50 μl of ^{125}I-labeled GnRH agonist [(D-Ala^6, N^{α}-MeLeu^7,Pro^{9}NEt)-GnRH, 60,000 to 70,000 count/min, from J. E. Rivier)] were added to 400 μl of appropriately diluted human pituitary membrane suspension, incubated for 150 minutes at 4°C, and filtered through Whatman GF/C filters. Total binding was determined in the absence of unlabeled peptide and nonspecific binding by the addition of $10^{-6}M$ GnRH agonist. The specific activity, as determined by self-displacement in the rat pituitary receptor assay, was 959 to 1066 μCi/μg.

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