STRUCTURE-ACTIVITY RELATIONSHIPS IN THE C-TERMINAL PART OF LUTEINIZING HORMONE RELEASING HORMONE(LH-RH)

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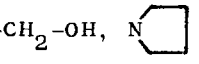
SUMMARY: Five new analogs of LH-RH(pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2), (Des-Gly-NH_2^{10}, Pro-methylamide^9)-LH-RH, (Des-Gly-NH_2^{10}, Pro-propylamide^9)-LH-RH, (Des-Gly-NH_2^{10}, Pro-ethanolamide^9)-LH-RH, (Des-Gly-NH_2^{10}, Pro-pyrrolidineamide^9)-LH-RH and (Des-Gly-NH_2^{10}, Pro-morpholineamide^9)-LH-RH were synthesized and evaluated for the hormonal activity. The activities of these new analogs were compared with those of LH-RH and our previously reported highly potent analog, (Des-Gly-NH_2^{10}, Pro-ethylamide^9)-LH-RH. The results demonstrate a significant contribution of the total chain-length of the hormone for the hormonal action.

Since the elucidation of the amino acid sequence of porcine hypothalamic luteinizing hormone releasing hormone (LH-RH, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) (1), several syntheses of LH-RH analogs have been described and the structure-activity relationships discussed (2). With regard to the C-terminal part of the molecule, Folkers et al. (3) reported that the Des-amide-LH-RH(LH-RH-decapeptide-OH) had very low specific activity, and Rivier et al. (4) reported the Des-Gly^{10}-analog of LH-RH to have approximately 10% the activity of LH-RH itself. Yanaihara et al. (5) reported that the synthetic analog, (Pro^8, Arg^9)-LH-RH, had no significant ac-

tivity. We subsequently reported the synthesis and biological properties of (Des-Gly-NH_2^{10}, Pro-ethylamide^9)-LH-RH /I/. This analog was extremely potent, over 5 times as active as LH-RH itself, in the ovulation-inducing assay (6).

These results led us to more detailed studies of the structure-activity relations in the C-terminal part of this hormone.

This paper deals with the synthesis and biological activities of five new analogs in which the Gly-NH_2^{10} was replaced by alkylamines: (Des-Gly-NH_2^{10}, Pro-methylamide^9)-LH-RH /II/, (Des-Gly-NH_2^{10}, Pro-propylamide^9)-LH-RH /III/, (Des-Gly-NH_2^{10}, Pro-ethanolamide^9)-LH-RH /IV/, (Des-Gly-NH_2^{10}, Pro-pyrrolidineamide^9)-LH-RH /V/ and (Des-Gly-NH_2^{10}, Pro-morpholineamide^9)-LH-RH /VI/.

Synthesis of peptides: For the synthesis of these analogs, the key intermediates, H-Leu-Arg(NO_2)-Pro-R(R=NH-CH_3, NH-CH_2-CH_2-CH_3, NH-CH_2-CH_2-OH,  and), were prepared by the conventional stepwise manner using the corresponding amino acid activated esters. The resulting intermediates were coupled with the N-terminal hexapeptide, pGlu-His-Trp-Ser-Tyr-Gly-OH (7), to yield the protected nonapeptide-alkylamides which were then treated with hydrogen fluoride (8) for removal of the nitro group in the molecules. The resulting crude peptides were purified by column chromatography on Amberlite XAD-2 and subsequently on carboxymethylcellulose in a manner similar to that described for LH-RH (7).

All peptide analogs thus obtained were chromatographically pure in three solvent systems (tlc, Woelm pre-coated silica gel F 254/366), gave the correct amino acid ratios and reasonable UV spectra. The data for characterization of the intermediates

TABLE I. PROPERTIES OF INTERMEDIARY PROTECTED PEPTIDES

Compound	$[\alpha]_D^{23}$	Rf(tlc)*	Elementary analysis (Calcd./Found)		
			C	H	N
Z-Leu-Arg(NO_2)-Pro-NH-CH_3	-41.5° (c=1.0 in MeOH)	0.25	$C_{26}H_{40}O_7N_8$:		
			54.15	7.02	19.43
			54.37	6.98	19.52
Z-Leu-Arg(NO_2)-Pro-NH-CH_2-CH_2-CH_3	-56.1° (c=1.0 in EtOH)	0.60	$C_{28}H_{44}O_7N_8$:		
			55.61	7.33	18.53
			55.63	7.56	18.58
Z-Leu-Arg(NO_2)-Pro-NH-CH_2-CH_2-OH	-46.6° (c=0.5 in EtOH)	0.49	$C_{27}H_{42}O_8N_8$:		
			52.67	7.04	18.20
			52.70	6.94	18.34
d-IBOC**-Leu-Arg(NO_2)-Pro-N⟨ ⟩	-53.6° (c=0.98 in MeOH)	0.62	$C_{32}H_{54}O_7N_8 \cdot H_2O$:		
			56.45	8.29	16.46
			56.97	8.17	15.75
Z-Leu-Arg(NO_2)-Pro-N⟨ ⟩O	-35.2° (c=0.5 in EtOH)	0.56	$C_{29}H_{44}O_8N_8 \cdot H_2O$:		
			53.52	7.14	17.22
			53.94	6.85	17.43

* Rf value (silica gel G) refers to the system: $CHCl_3$:MeOH:AcOH (9 : 1 : 0.5)

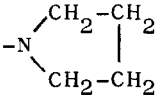
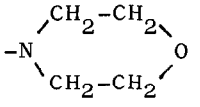
** d-IBOC-: d-Isobornyloxycarbonyl-.

and the analogs are listed in Table I and II, respectively.

Hormonal evaluation: The ovulation-inducing activity of these nonapeptide analogs were performed by using adult female rats as described by Yamazaki and Nakayama (9). LH and FSH release activity *in vitro* of these analogs in hemisected male rat pituitaries were also assayed by the Parlow (10) and Steelman-Pohley (11) methods and/or the radioimmunoassay for LH of Niswender et al. (12) as described by White et al. (13) and the radioimmunoassay for FSH employing the NIAMD-Rat-FSH-Kit. The data on the biological activities of the synthetic LH-RH analogs are listed in Table III.

DISCUSSION: The structure and the biological activity of our new nonapeptide amide derivatives and of the structurally

related analogs, which have already been reported (3,4,6), can be summarized as follows:

Compound	Structure	Hormonal activity
LH-RH	pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NH-CH_2-CO-NH_2	100%
LH-RH-OH (Folkers et al.[3])	-NH-CH_2-COOH	0.1%
(Des-Gly^{10})-LH-RH (River et al.[4])	-NH_2	11%
Analog II	-NH-CH_3	80-100%
Analog I	-NH-CH_2-CH_3	500%
Analog III	-NH-CH_2-CH_2-CH_3	200-300%
Analog IV	-NH-CH_2-CH_2-OH	100-150%
Analog V		70-80%
Analog VI		20-30%

Although it seems somewhat premature to conclude the structure-activity relations from these limited number of analogs, the terminal glycine amide does not appear to be essential for the high level of the hormonal activity for release of LH. Our data also suggest that the total chain-length of this hormone molecule might have a very important role in the binding the hormone to its receptor(s) at the target organ, pituitary. Moreover, the low activity of LH-RH-OH could be explained as a result of a poor binding of the negative charge at the terminal carboxylic acid of this analog.

TABLE II. CHEMICAL AND PHYSICAL PROPERTIES OF LH-RH ANALOGS

Compound*	Rf** a	b	c	$[\alpha]_D^{24}$ (c=0.5 in 5% AcOH)	Amino acid analysis***
I	0.43	0.072	0.51	-56.2°	His 0.95, Arg 0.98, Glu 0.98, Ser 0.95, Pro 1.00, Gly 1.00, Leu 0.99, Tyr 1.00, ethylamine 1.10, Trp 0.94
II	0.36	0.048	0.49	-55.6°	His 0.96, Arg 0.98, Ser 0.96, Glu 1.00, Pro 1.00, Gly 1.00, Leu 0.98, Tyr 0.93, methylamine 1.12, Trp 1.10
III	0.47	0.083	0.54	-58.8°	His 0.81, Arg 0.97, Ser 0.92, Glu 1.00, Pro 0.97, Gly 1.03, Leu 1.05, Try 1.03, propylamine 0.97, Trp 0.98
IV	0.34	0.036	0.46	-54.4°	His 1.00, Arg 1.05, Ser 0.95, Glu 1.00, Pro 1.05, Gly 1.00, Leu 0.95, Tyr 0.76, ethanolamine 0.96, Trp 0.98
V	0.38	0.048	0.49	-56.4°	His 1.09, Arg 0.96, Ser 0.82, Glu 1.00, Pro 1.05, Gly 1.00, Leu 0.95, Tyr 0.96, Trp 1.02
VI	0.34	0.048	0.48	-66.6°	His 1.00, Arg 1.05, Ser 1.00, Gly 1.00, Pro 1.15, Gly 1.00, Leu 1.05, Tyr 0.90, Trp 1.14

* The systematic names of these compounds are in the text.

** Rf a, b and c value refers to the systems: n-BuOH:AcOH:EtOAc:H_2O (1:1:1:1); n-BuOH:AcOH:H_2O (4:1:1); n-BuOH:Pyridine:AcOH:H_2O (30:20:6:24), respectively.

*** The Trp content were calculated from UV spectrum. The average amino acid recovaries of all the peptides are 86 ± 2%.

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TABLE III. BIOLOGICAL ACTIVITY OF LH-RH ANALOGS

OVULATION-INDUCING ACTIVITY (rat)

Dosage (ng/100 g b.w.*)	Compound							
	LH-RH		/II/	/I/	/III/	/IV/	/V/	/VI/
	(No. of ovulating rats/No. of examined rats)							
10				0/10				
20				1/10				
30				5/10	0/10			
40				6/10	3/10			
50		0/10		9/10	4/10	0/5		
60					5/10			
80					9/10			
100	1/15	2/10	1/5	4/4		4/10		0/5
200	3/20	7/10	3/5			5/10	1/5	
300	14/20	10/14		7/7		5/7	4/5	0/5
400	18/20	10/10					5/5	
600								1/5
1000			4/4	3/3		3/3	3/3	3/3

in vitro

LH-release	100 (%) (P, RIA)	25-75(%) (P)	250-300(%) (P, RIA)	135(%) (P)	132-280(%) (P, RIA)	35-80(%) (P, RIA)	20(%) (P)
FSH-release	100 (%) (SP, RIA)	-	250-575(%) (SP,RIA)	150(%) (SP)	190-250(%) (SP,RIA)	60(%) (SP)	20(%) (SP)

* b.w. = body weight.

In the lower section of the Table, the following abbreviations are used: P = Parlow's method, SP = Steelman-Pohley's method, RIA = for LH, method of Niswender et al. (OO-Rat-RIA); for FSH, NIAMD-Rat-FSH-Kit.

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