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THE DOMINANT ROLE OF AMIDE GROUP AT C-TERMINUS FOR RECOGNITION BY ANTIBODY IN PRIMATES AGAINST GONADOTROPIN RELEASING HORMONE

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Summary—A monkey and a baboon immunized with GnRH-tetanus toxoid conjugate developed high anti-GnRH antibody titres which resulted in disruption of cyclicity and low estradiol and progesterone levels indicative of the inhibition of follicular development and ovulation. Sera of both animals reacted with GnRH(NH_2) but were devoid of reactivity with peptide sequences, 4–6, 7–10 and 4–10 of GnRH as well as GnRH free acid. Both sera were however reactive with GnRH-Lys-muramyl dipeptide analogue and GnRH-Ala-Ala-Thr-Lys-Pro-Arg-OH. As these compounds differ from GnRH-free acid by the presence of amide linkage at C-terminus of GnRH, these studies point to the importance of the conformation involving amide group at this position for immunoreactivity.

INTRODUCTION

This paper reports experimental studies on two sera raised in a monkey and in a baboon by immunization with gonadotropin-releasing hormone (GnRH) linked to tetanus toxoid (TT) by a procedure described elsewhere [1]. Immunizations were carried out without the use of Freund's complete adjuvant (FCA), employing sodium phthalyl derivative of lipopolysaccharide (SPLPS) or muramyl dipeptide analogue [MDP] 1[2-(2-hexamido-2-deoxy-D-glucose-3-oxyl)-D-propionyl-L-alanyl-D-isoglutamine]. Booster injections were given with an innocuous lipidic emulsion, LBA (Leiras Basic Adjuvant, available from Leiras Pharmaceutical Plant, Hubtamaki Oy, Turku, Finland). The immunization by this procedure produced high enough antibody titres to abolish the normal cyclicity of the animal [2]. The duration of the response was fairly long e.g. 80 weeks [2]. The plasma progesterone values were low in confirming the bioefficacy of these antibodies [2].

EXPERIMENTAL

Highly purified native GnRH(NH_2) (lot IBR-5483) and GnRH free acid [GnRH(OH)] were made available by Population Council, New York. The tripeptide (4–6), tetrapeptide (7–10) and heptapeptide (4–10) of GnRH were synthesized by classical solution phase methodology as described [3]. GnRH-Lys-MDP was synthesized by the method described by Carelli *et al.*[4]. GnRH-Ala-Ala-Thr-Lys-Pro-Arg-OH was synthesized by solid phase methodology as per Chaudhuri *et al.*[5]. The radioiodination of GnRH was carried out by IODO-GEN method [6]. The cross-reaction of GnRH fragments and GnRH derivative was studied by competitive inhibition of the binding of ^{125}I-labelled GnRH [3].

RESULTS AND DISCUSSION

Figure 1 summarizes the salient binding characteristics of these antibodies. A remarkable feature was the identity of the properties of antibodies in both cases. The association constant (K_a) of antibodies for GnRH was in both animals of the order of 10^{10}–10^{11} l/mol. While both sera reacted well with native GnRH in which glycine at position 10 was an amide group and not a free carboxyl group (—COOH), the reactivity of both sera was zero with GnRH(OH) (Fig. 1). The sera on the other hand recognized to some extent GnRH-Ala-Ala-Thr-Lys-Pro-Arg-OH and GnRH-Lys-MDP (Fig. 1). An apparent difference between these peptides and GnRH(OH) is the presence of an amide linkage at the C-terminus. The Figure also gives data on the lack of competition obtained with the tripeptide (4–6), tetrapeptide (7–10) and heptapeptide (4–10). The amounts of the different peptides needed for achieving 50% inhibition of binding of native GnRH to anti-GnRH antisera are given in Table 1.

The loss of immunoreactivity in the absence of the C-terminal amide may result from the negative charge introduced by the —COOH group, which in turn creates a conformation of the molecule that does not fit in the antibody combining site. Alternatively, it can be assumed that the amide group at or near the amino acid 10 contributes in an important way to the immunological determinant(s). The introduction of an amide group at a site adjacent to amino acid

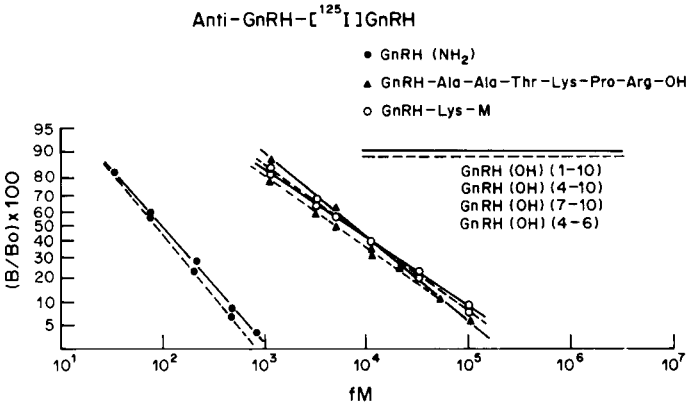

Fig. 1. Binding characteristics of bioeffective antibodies from a monkey and baboon. Cross reaction was studied by competitive inhibition of the binding of [^{125}I]GnRH to the antisera[10]. The assay system consisted of the following: 0.02 ml normal horse serum, 0.05; [^{125}I]GnRH, 0.05 ml of various competitors of varying concentration and 0.05 ml antiserum at appropriate dilution to give 30–50% binding in absence of competitors. The inhibition lines were obtained by plotting the logit of B/Bo against log dose antigen. B = reactivity bound in presence of labelled GnRH and competing unlabelled peptide. Bo = radioactivity bound with labelled GnRH alone. Values are expressed in moles. The dotted line data indicate experiment with monkey serum while solid line shows the data of baboon.

Table 1. Amount of peptide for 50% inhibition of the binding of [^{125}I]GnRH to the anti-GnRH antibodies

Peptide	ED50(in M)		Relative Reactivity $\times 10^3$	
	Monkey	Baboon	Monkey	Baboon
p-Glu-His-Try-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2	8.0×10^{-14}	9.5×10^{-14}	1000	1000
H-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-OH	—	—	0	0
H-Leu-Arg-Pro-Gly-OH	—	—	0	0
H-Ser-Tyr-Gly-OH	—	—	0	0
p-Glu-His-Tyr-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-OH	—	—	0	0
p-Glu-His-Tyr-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-Lys-MDP	6.9×10^{-12}	6.8×10^{-12}	11.59	13.97
p-Glu-His-Try-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-Ala-Ala-Thr-Lys-Pro-Arg-OH	4.8×10^{-12}	7.2×10^{-12}	16.96	13.19

10, as is the case in conjugates GnRH-Lys-MDP or GnRH-Ala-Ala-Thr-Lys-Pro-Arg-OH, restored partly the regain of the parental conformation of the molecule with which these antibodies react.

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