Differential Effects of Resact Analogues on Sperm Respiration Rates and Cyclic Nucleotide Concentrations†

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ABSTRACT: Analogues of resact (Cys-Val-Thr-Gly-Ala-Pro-Gly-Cys-Val-Gly-Gly-Gly-Arg-LeuNH_2) were synthesized to determine whether or not a stimulation of sperm respiration could be obtained independent of elevations of cyclic nucleotide concentrations. Modification of the CO_2-terminal leucine NH_2 did not alter biological activity; however, substitution of the two cysteinyl residues by Ser or Tyr or methylation of the cysteinyl residues resulted in divergent relative potencies dependent on whether respiration rates or cyclic nucleotide concentrations were measured. [Ser[1],Tyr[8]]resact, as an example, was approximately 40% as potent as resact at stimulating respiration rates but was 1% as effective as resact at causing cyclic GMP elevations. An NH_2-terminal fragment (Cys-Val-Thr-Gly-Ala-Pro-Gly) neither stimulated respiration nor elevated cyclic nucleotide levels at concentrations up to 10 μM whereas a CO_2-terminal fragment (Cys-Val-Gly-Gly-Gly-Arg-LeuNH_2) had approximately 20% of the respiration activity and 0.1% of the cyclic GMP elevating activity of resact. When the CO_2- and NH_2-terminal fragments were added simultaneously, however, cyclic nucleotide concentrations were elevated at the same relative concentrations as observed with resact. An analogue ([^{125}I-[Tyr[1],Ser[8]]resact) was subsequently synthesized and used for receptor binding studies. Both the NH_2-terminal and CO_2-terminal fragments competed for binding, although they were 0.0004 and 0.025 times as effective as resact, respectively. However, in the presence of 1 μM resact-(1–7), resact-(8–14) was almost as potent as resact in the competitive binding assay. The individual CO_2- and NH_2-terminal fragments appear to each bind to receptor, therefore, but the presence of one greatly potentiates the binding of the other peptide fragment. It also appears that cyclic nucleotide elevations can be separated from the stimulation of respiration, suggestive that increased concentrations of cyclic AMP or cyclic GMP do not mediate the effects of the peptides on respiration.

Eggs and/or secretions of the female reproductive tract from many different animals, including the human, appear to stimulate spermatozoan metabolism and motility (Garbers & Kopf, 1980). In mammals, a hyperactivated form of sperm motility has been observed in rabbits, guinea pigs, mice, bats, dolphins, hamsters, dogs, cattle, sheep, and humans (Yanagimachi, 1970, 1972; Phillips, 1972; Barros et al., 1973; Yanagimachi & Usui, 1974; Mahi & Yanagimachi, 1976; Cooper et al., 1979; Aonuma et al., 1980; Johnson et al., 1980; Lambert, 1981; Cummins, 1982; Fleming et al., 1982; Mortimer et al., 1983); therefore, if metabolic or motility activation is important for fertilization, intervention may have agricultural or medical applications. In our laboratory, two classes of peptides have been isolated from sea urchin eggs that stimulate sperm respiration rates and motility (Hansbrough & Garbers, 1981; Garbers et al., 1982; Suzuki et al., 1984). A peptide isolated from *Arbacia punctulata* has been named resact (Cys-Val-Thr-Gly-Ala-Pro-Gly-Cys-Val-Gly-Gly-Gly-Arg-LeuNH_2), and a peptide obtained from *Strongylocentrotus purpuratus* has been called speract (Gly-Phe-Asp-Leu-Asn-Gly-Gly-Gly-Val-Gly) (Hansbrough & Garbers, 1981a; Garbers et al., 1982; Suzuki et al., 1984). Speract also has been isolated from *Hemicentrotus pulcherrimus* eggs (Suzuki et al., 1981). These peptides (speract and resact) increase sperm cyclic AMP and cyclic GMP concentrations in a species-specific manner (Suzuki et al., 1984; Suzuki & Garbers, 1984; Ramarao & Garbers, 1985). Recent evidence also suggests that resact is a potent chemoattractant (Ward et al., 1985). Resact and speract reduce the activity of guanylate cyclase under certain assay conditions and cause the formation of a newly stained membrane protein as assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Suzuki et al., 1984; Ramaro & Garbers, 1985).

Structure/function relationships of resact have not been evaluated, although such studies may be of importance in the analysis of the biochemical mechanism of action of these peptides as well as of use in defining the biological significance of the peptides. Here, various analogues of resact have been synthesized, and the effects of structural changes on respiration-stimulating, cyclic nucleotide elevating, and receptor-binding properties are determined.

Experimental Procedures

Materials. A. punctulata were obtained from the Marine Biological Laboratory, Woods Hole, MA, and from Gulf Specimen Co., Panama, FL. Sea water was prepared in the laboratory to contain 454 mM NaCl, 9.7 mM KCl, 24.9 mM $MgCl_2$, 9.6 mM $CaCl_2$, 27.1 mM $MgSO_4$, 4.4 mM $NaHCO_3$, and 10 mM ACES (pH 6.6). *N-tert*-Butoxycarbonyl L-amino acids and *N-tert*-butoxycarbonyl L-amino acid resins were from Peninsula Laboratories, San Carlos, CA. Other reagents of the highest grade available were purchased from Sigma Chemical Co. or Pierce Chemical Co.

Amino Acid Analysis. Amino acid analysis was performed in a LKB 4400 amino acid analyzer and/or by a PICO-TAG precolumn derivatization method after 22~24-h hydrolysis in 6 N HCl at 110–115 °C under vacuum.

Peptide Synthesis. Peptide synthesis was performed with a system 990 synthesizer (Beckman Instruments, Inc., Berkeley, CA), starting with the Merrifield resin (Erickson & Merrifield, 1976). After sequential addition of the intended

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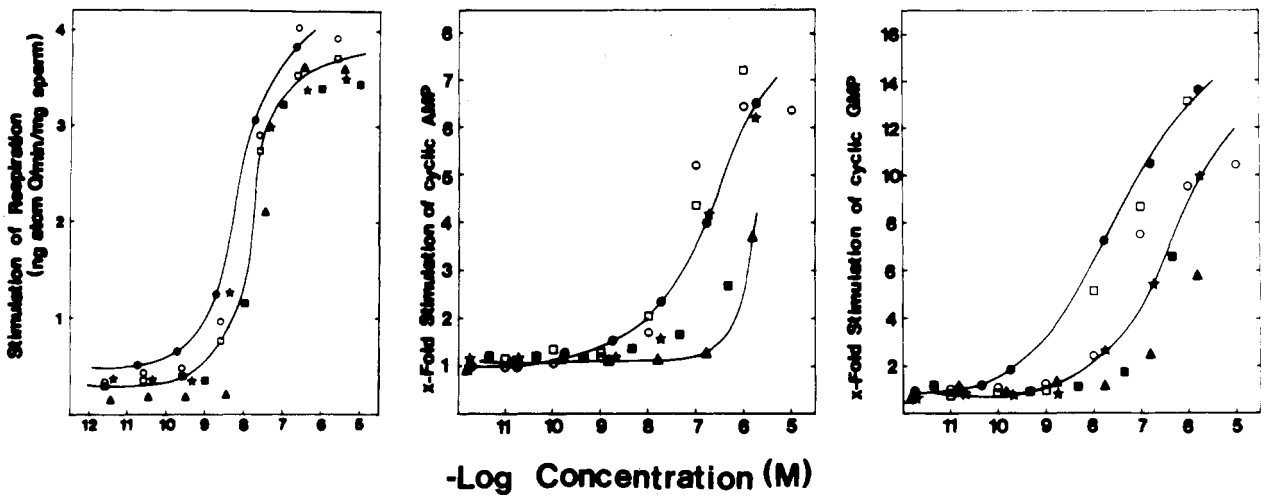

FIGURE 1: Stimulation of respiration and elevation of cyclic nucleotide concentrations by resact and cysteinyl analogues of resact. Cyclic nucleotide concentrations were estimated at 15 s after addition of peptide. Resact (●); [Tyr[1],Ser[8]]resact (○); [Ser[1],Tyr[8]]resact (▲); [Tyr[1],Cys(Me)[8]]resact (■); [Cys(Me)[1],Tyr[8]]resact (*); [Cys(Me)[1],Cys(Me)[8]]resact (□).

amino acids, the peptide was removed from the resin with HF in the presence of anisole and was then purified by high-performance liquid chromatography (HPLC). Peptides were generally purified on octyl resins (HPLC) by the use of acetonitrile (0.1% trifluoroacetic acid) gradients. Following purification, analytical thin-layer chromatography [silica gel with solvents containing 1-butanol/acetic acid/pyridine/H_2O (15:3:10:12) or acetic acid/pyridine/formic acid/H_2O (63:21:20:6)], paper electrophoresis, and repeated HPLC (C8 or C18 reverse phase) confirmed the purity of each peptide. The proper composition was then established by amino acid analysis.

Esterification of Peptide. Into a solution of [Leu[14]]resact in methyl alcohol or ethyl alcohol, one drop of concentrated hydrochloric acid was added, and the solution was then kept at room temperature for 3 h. After neutralization with $NaHCO_3$, the solution was applied to reverse-phase high-performance liquid chromatography columns for purification.

Respiration Rates. Respiration rates of spermatozoa were determined at pH 6.6 as previously described (Hansbrough & Garbers, 1981a).

Cyclic AMP and Cyclic GMP. The concentrations of cyclic AMP and cyclic GMP in the sperm cells were measured as described previously (Hansbrough & Garbers, 1981a,b). To accentuate cyclic nucleotide responses, all incubation mixtures contained 50 μM 1-methyl-3-isobutylxanthine. Cyclic nucleotide concentrations were generally measured at 15 s after the addition of each peptide to spermatozoa. Basal (nonstimulated) concentrations of cyclic AMP ranged from 2 to 4 nmol/g wet weight and of cyclic GMP from 0.3 to 0.5 nmol/g wet weight in the presence of 1-methyl-3-isobutylxanthine.

Iodination. Iodination of [Tyr[1],Ser[8]]resact was accomplished by the chloramine T method (Hunter & Greenwood, 1962). In general, a solution of peptide (2 nmol) in water (15 μL) and Na[125]I (1 mCi) were mixed with a solution of 3.5 mM chloramine T in 0.15 M sodium phosphate buffer (pH 7.5) (5 μL) and incubated for 1 min. This was followed by the addition of 6 mM sodium metabisulfite in the same buffer (5 μL). The mixture was applied to a Bio-Gel P-6 column (1 × 50 cm) equilibrated with 0.1 M sodium borate buffer (pH 8.5) containing 0.1% gelatin. The iodinated peptide was separated from the [125]I-labeled reagent and was shown to be >95% pure by chromatography on reverse-phase HPLC.

Binding Studies. Sperm cells (30 μg wet weight) were incubated with [125]I-[Tyr[1],Ser[8]]resact (20 000~30 000 cpm) at 17 °C for 30 min in sea water (pH 6.6) (1 mL) containing 1 mg/mL bovine serum albumin. Approximately 8 × 10^{10} cells are equivalent to 1 g wet weight. The reaction was stopped by dilution with cold sea water (4 mL) and immediate filtration on Whatman GF/C filters. After being rinsed with cold sea water (5 mL) 3 times, the dried filters were counted for radioactivity. Nonspecific binding was defined as the binding not competed for by 5.4 nmol of nonradioactive resact.

RESULTS

Leucine Derivatives of Resact. Three CO_2-terminal leucine analogues of resact ([Leu[14]]resact, [Leu(Me)[14]]resact, [Leu(Et)[14]]resact) stimulated respiration and elevated cyclic nucleotide concentrations at the same concentrations as resact (not shown). Therefore, the amide form of resact is not required for biological activity.

Cysteinyl Analogues of Resact. A repetitive sequence of Cys-Val-X-Gly occurs twice in resact. The cysteinyl residues were substituted with Ser or Tyr residues and/or were protected with methyl groups. The various analogues were from about 20 to 70% as effective as resact on respiration (Figure 1). However, with respect to cyclic nucleotide elevations, greater differences in relative potencies of the analogues were observed. A number of analogues ([Tyr[1],Ser[8]]resact, [Cys(Me)[1],Cys(Me)[8]]resact, and [Cys(Me)[1],Tyr[8]]resact) had the same apparent relative potency as resact with respect to elevations of cyclic AMP whereas two analogues ([Tyr[1],Cys(Me)[8]]resact and [Ser[1],Tyr[8]]resact) were 10% or less as potent as resact (Figure 1). With respect to elevations of cyclic GMP, most analogues were less potent than resact; the [Ser[1],Tyr[8]]resact analogue required over 100-fold higher concentrations than resact for half-maximal elevations of cyclic GMP (Figure 1). From these results, it can be suggested that the stimulation of respiration rates is not necessarily coupled to cyclic nucleotide elevations; the question also can be raised as to whether or not different populations of peptide receptor exist on the spermatozoa.

CO_2- and NH_2-Terminal Fragments. The two fragments resact-(1–7) and resact-(8–14) proved especially valuable for defining structure/activity relationships. The NH_2-terminal peptide [resact-(1–7)] failed to stimulate respiration rates or to elevate cyclic nucleotide concentrations at any concentration tested, while the CO_2-terminal fragment elevated respiration rates half-maximally at 40 nM compared to values of 6–12 nM for resact (Figure 2). However, the CO_2-terminal

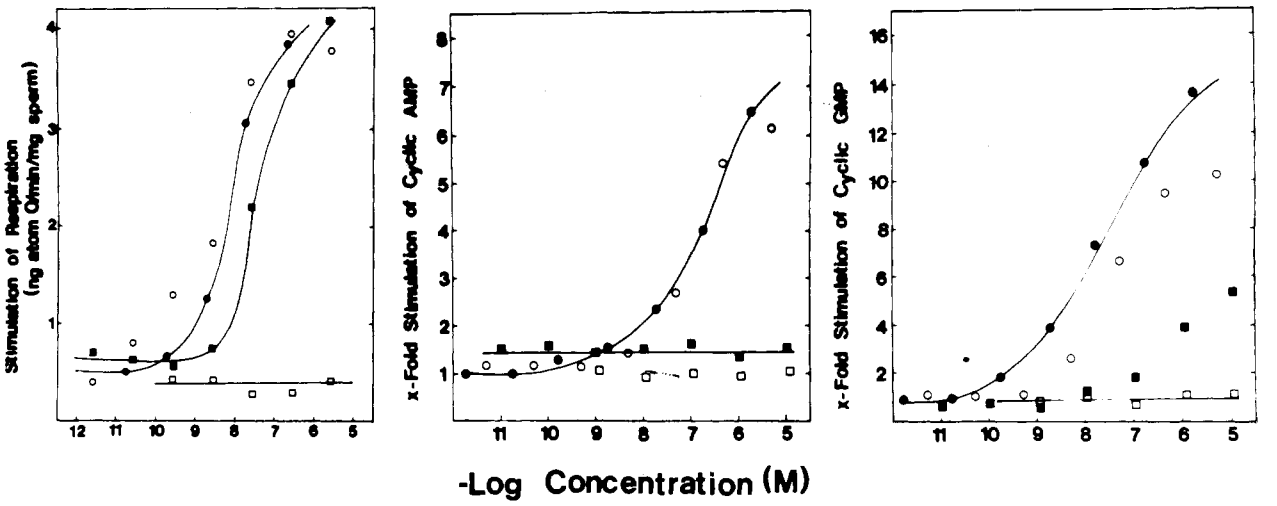

FIGURE 2: Stimulation of respiration rates and the elevation of cyclic nucleotide concentrations by an elongated resact analogue and by resact-(1–7) and resact-(8–14). Cyclic nucleotide concentrations were estimated at 15 s except with resact-(8–14) where concentrations were estimated at 5 min. Resact (●); Gly-Gly-Gly-(Tyr[1],Ser[8])resact (○); resact-(1–7) (□); resact-(8–14) (■).

fragment failed to cause detectable elevations of cyclic AMP at any concentration tested and elevated cyclic GMP half-maximally only at greater than 1000-fold higher concentrations than resact (Figure 2). The relative potency differences remained the same whether cyclic nucleotides were estimated at 15 s or at 5 min in the cells treated with the peptide fragments. Therefore, a markedly divergent response to the peptides with respect to cyclic nucleotide elevation and respiration stimulation was evident and different structural features were required for either response. The elevations of cyclic GMP caused by resact-(8–14) also had a different time course than those induced by resact (Figure 3). A transient large peak in cyclic GMP elevations induced by resact was repeatedly absent in response to resact-(8–14).

Extension of NH_2 Terminus. The peptide Gly-Gly-Gly-[Tyr[1],Ser[8]]resact had the same respiration-stimulating activity as resact (Figure 2). Therefore, extension of the NH_2 terminus had a small positive effect as compared to [Tyr[1],Ser[8]]resact (see Figure 1). The elongated peptide continued to be slightly less effective than resact at causing elevations of cyclic GMP concentrations (Figure 2).

Combinations of CO_2- and NH_2-Terminal Fragments. Combinations of resact-(1–7) and resact-(8–14) had unexpected effects on the spermatozoa. When the NH_2-terminal fragment concentration was varied at a fixed concentration of resact-(8–14) or when the CO_2-terminal fragment was varied at a fixed concentration of resact-(1–7), both cyclic AMP and cyclic GMP concentrations were elevated half-maximally by each fragment at concentrations similar to that found with intact resact (Figure 4). Additionally, a transient peak of cyclic GMP observed at 15 s or less was again observed when the fragments were added together (data not shown). The same transient peak in cyclic AMP concentrations also was observed when the fragments were added together (data not shown).

Receptor Binding. The [Tyr[1],Ser[8]]resact analogue stimulated respiration only slightly less effectively than resact and had equivalent potency with respect to elevations of cyclic AMP. The [125]I-labeled peptide was specifically bound to intact spermatozoa with an equilibrium in binding being reached by approximately 30 min (not shown); nonspecific binding was less than 5%. The binding of [125]I-[Tyr[1],Ser[8]]resact to spermatozoa was dependent on cell concentration, with linearity observed between 10 and 50 μg (wet weight) of cells/mL (Figure 5). Speract, a peptide obtained from *S. purpuratus*

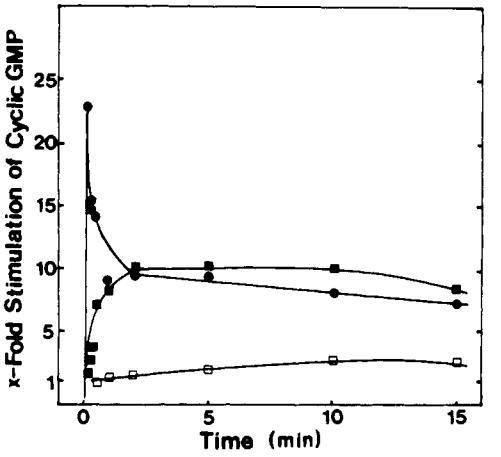

FIGURE 3: Time course of cyclic GMP elevations in response to resact, resact-(1–7), or resact-(8–14). Resact (1.6 μM), resact-(1–7) (10 μM), or resact-(8–14) (10 μM) was added to spermatozoa, and reactions were then stopped at the times indicated on the abscissa with 0.5 N perchloric acid. Resact (●); resact-(8–14) (■); resact-(1–7) (□).

eggs that does not stimulate *A. punctulata* spermatozoa, did not compete with radiolabel (not shown). In studies not shown, it was determined that there are approximately 14 000 receptors/cell; Scatchard plots appeared linear with an estimated K_d = 1 nM.

Resact and analogues of resact were subsequently tested as competitors of [125]I-[Tyr[1],Ser[8]]resact binding. With respect to the leucine derivatives, the carboxylic acid and ester analogues competed with the radiolabeled resact analogue about 0.1 times as effectively as resact (data not shown), despite apparent equivalent respiration-stimulating activity.

The cysteinyl derivatives of resact competed for [125]I-[Tyr[1],Ser[8]]resact binding with a wide range of relative potencies (Figure 6). [Tyr[1],Ser[8]]resact was about 0.005 times as potent as resact despite similar potency with respect to respiration and cyclic AMP elevations. The most ineffective analogue was [Tyr[1],Cys(Me)[8]]resact, which was also one of the least effective analogues at causing elevations of cyclic AMP or cyclic GMP. The elongated resact analogue and resact were approximately equipotent, causing half-maximal decreases in bound radioactivity at 10 nM (Figure 7). Resact-(8–14) and resact-(1–7) required about 80 and >10 000 times higher concentrations than resact, respectively, for

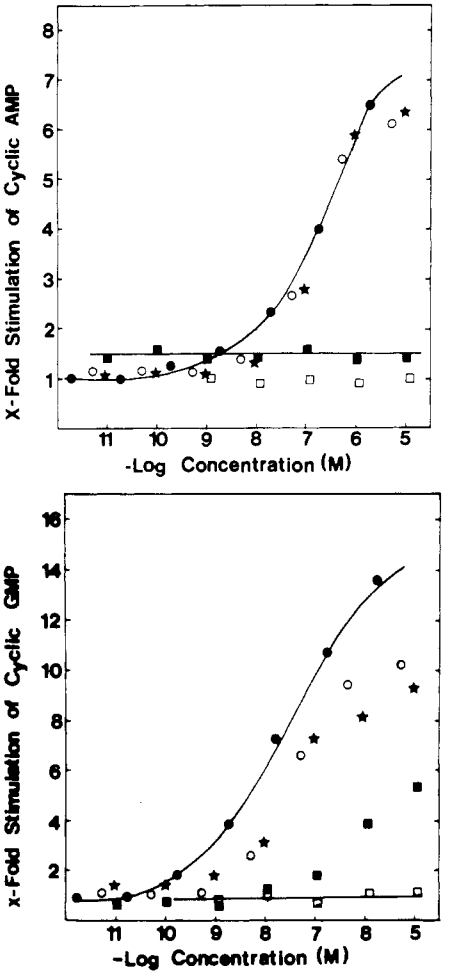

FIGURE 4: Elevation of cyclic nucleotide concentrations by resact, resact-(1–7), resact-(8–14), or a combination of the fragments. Resact (●), resact-(1–7) (□), or resact-(8–14) (■) were varied as usual. However, in some experiments resact-(1–7) was kept constant at 10 μM and resact-(8–14) was then varied (*) while in other experiments resact-(8–14) was maintained constant at 1 μM while resact-(1–7) was varied (○). Cyclic nucleotide concentrations were estimated at 15 s for resact and at 5 min for the peptide fragments.

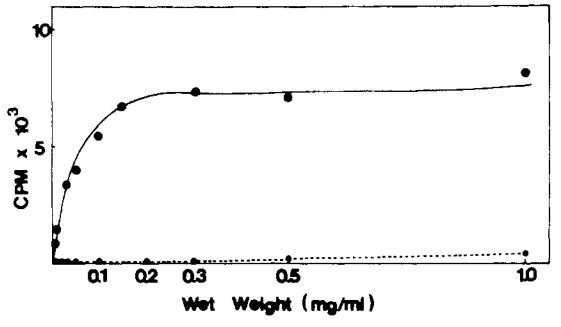

FIGURE 5: Binding of ^{125}I-[Tyr^1,Ser^8]resact to spermatozoa as a function of cell concentration. Approximately 400 fmol of the ^{125}I-labeled analogue was added to each assay reaction. The binding reactions were performed as described under Experimental Procedures for 30 min. The lower line represents the binding of radiolabel in the presence of 5.4 nmol of nonradioactive resact.

equivalent competitive binding with radiolabel.

Resact or a mixture of the two fragments [resact-(1–7)and resact-(8–14)], each at half the total peptide concentration, competed equally effectively with the radiolabeled peptide. When resact-(1–7) was kept constant at 1 μM (a concentration that did not compete for radiolabel) and resact-(8–14) was varied, the fragment required about 4 times higher concentrations than resact for a 50% reduction in specific binding

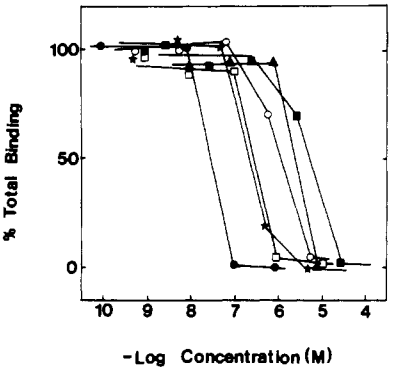

FIGURE 6: Ability of resact and substituted analogues of resact to compete with ^{125}I-[Tyr^1,Ser^8]resact for binding to sperm cells. The data presented are the average of three experiments and are expressed as percent binding of control. Resact (●); [Tyr^1,Ser^8]resact (○); [Ser^1,Tyr^8]resact (▲); [Tyr^1,Cys(Me)^8]resact (■); [Cys-(Me)^1,Tyr^8]resact (*); [Cys(Me)^1,Cys(Me)^8]resact (□).

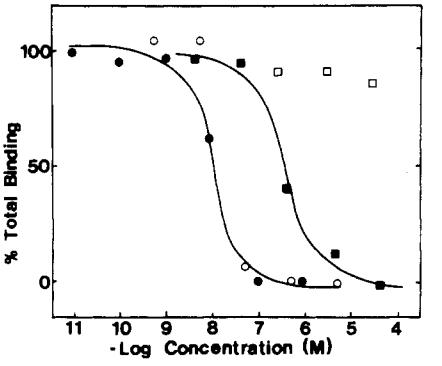

FIGURE 7: Ability of resact and substituted analogues of resact to compete with ^{125}I-[Tyr^1,Ser^8]resact for binding to sperm cells. The data presented are the average of three experiments and are expressed as percent binding of control. Resact (●); Gly-Gly-Gly-[Tyr^1,Ser^8]resact (○); resact-(8–14) (■); resact-(1–7) (□).

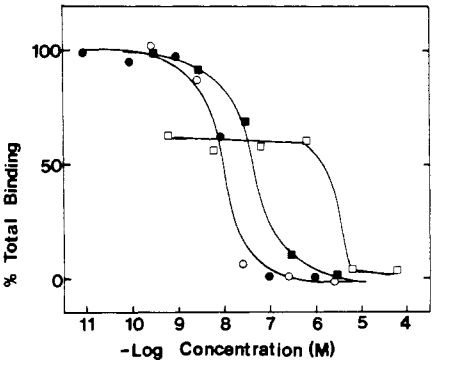

FIGURE 8: Ability of resact and the combination of resact-(1–7) and resact-(8–14) to compete with ^{125}I-[Tyr^1,Ser^8]resact for binding to sperm cells. Resact (●); combination of the two peptide fragments each at half the concentration given on the abscissa (○); variable resact-(8–14) at 1 μM resact-(1–7) (■); variable resact-(1–7) at 100 mM resact-(8–14) (□).

(Figure 8). When resact-(8–14) was kept constant at 100 nM (a concentration that reduced binding of radiolabel by about 40%) and resact-(1–7) was varied, the fragment required approximately 5.4 μM concentrations for 50% further reductions in radiolabel binding (Figure 8); this compares to concentrations of about 500 μM that are required in the absence of resact-(8–14).

Discussion

In this study three types of resact analogues were synthesized: (1) carboxylic acid derivatives of C-terminal leucine,

Table I: Approximate Half-Maximal Concentrations of Resact or Resact Analogues Required for Stimulation of Respiration Rates, Elevation of Cyclic Nucleotide Concentrations, or Competition with Radiolabel for Receptor Binding

peptide	concn required for half-maximal effects (nM)			
	respiration	cAMP	cGMP	competitive binding
resact	8	200	30	10
[Tyr^1,Ser^8]resact	55	200	100	4000
[Ser^1,Tyr^8]resact	18	1800	3000	7000
[Tyr^1,Cys(Me)^8]resact	25	1800	500	9000
[Cys(Me)^1,Tyr^8]resact	13	200	500	400
[Cys(Me)^1,Cys(Me^8]resact	18	200	80	500
resact-(1–7)	>10000	>50000	>500000	>400000
resact-(8–14)	40	>50000	30000	800
resact-(1–7) + 100 nM resact-(8–14)				5000
resact-(8–14) + 1 μM resact-(1–7)				40

(2) substituted and/or protected analogues at the 1- and 8-positions (Cys), and (3) peptides with altered numbers of amino acids. The biological activity and receptor binding properties of the analogues can be summarized as follows: (1) the amide form of resact is not essential for either respiration stimulation or cyclic nucleotide elevations, (2) the two cysteinyl residues can be substituted by other amino acids with retention of respiration-stimulating activity, (3) the modification of the cysteinyl residues results in analogues with different abilities to elevate cyclic nucleotide concentrations, (4) the seven CO_2-terminal amino acids are sufficient for the stimulation of respiration rates but cyclic nucleotide concentrations are elevated only at 1000-fold higher concentrations than resact, (5) the seven NH_2-terminal amino acids fail to stimulate respiration rates or to elevate cyclic nucleotide concentrations at concentrations as high as 10 μM, (6) the combination of the two separate peptide fragments results in almost the same biological potency with respect to cyclic nucleotide elevations as is observed with resact, and (7) the peptide fragments interact to stimulate the binding of each other. A summary of the approximate concentrations required for half-maximal effects on each of the parameters measured is given in Table I.

An explanation for the different structural requirements for respiration activation and cyclic nucleotide elevation remains to be determined. In studies on the membrane receptor for speract, Dangott and Garbers (1984) synthesized ^{125}I-[GGGY^2]speract and cross-linked the analogue to the receptor. Only one radiolabeled band was apparent upon sodium dodecyl sulfate gel electrophoresis, and its molecular weight was about 77000. If a single peptide receptor exists, the data obtained could be explained if the peptide binding site on the receptor has a distinct site for the CO_2 and NH_2 domains of resact. One site could be coupled to respiration activation whereas both sites would need to be occupied for maximal elevations of cyclic nucleotides. Alternatively, multiple receptors may exist. In the work of Dangott and Garbers (1984), low concentrations of the ^{125}I-labeled analogue were used in the cross-linking studies, and therefore, a low-affinity receptor still could be present.

The binding data obtained with resact and resact analogues demonstrate that relative potency is not correlated with any particular biological event. Resact is the most potent peptide in all cases and resact-(1–7) the least potent. In general, the relative potencies of peptides to elevate cyclic nucleotide concentrations, as opposed to respiration rates, are better correlated with binding data.

Since resact is a potent chemoattractant (Ward et al., 1985), the analogues should prove valuable in future studies on the mechanisms involved in directional motility. Resact is the first defined egg substance from an animal that has chemoattractant properties, although such behavior of spermatozoa in response to eggs and/or substances released from eggs has been well established (Miller, 1985). The directional response to resact requires extracellular Ca^{2+} (Ward et al., 1985); the Ca^{2+} requirement may not be at the level of receptor binding since speract binds to its receptor and stimulates respiration rates in the absence of added extracellular Ca^{2+} (Hansbrough & Garbers, 1981a,b; Smith & Garbers, 1983); therefore, an unidentified Ca^{2+}-dependent step appears to be present after receptor occupancy. In studies on speract analogues, Repaske and Garbers (1983) showed that independent of peptide structure a high positive correlation existed between net H^+ efflux and the stimulation of sperm respiration. These kinds of studies also will need to be done with the resact analogues to determine if net H^+ efflux continues to correlate with respiration changes or with cyclic nucleotide changes. Chemotactic responses of neutrophils (Goldman et al., 1985; Verghese et al., 1985; Okajima et al., 1985) and possibly even bacteria (Ordal, 1977; Stock et al., 1985) could involve similar mechanisms, and the sperm cells, therefore, may serve as a parallel model system for studies on the biochemical mechanisms involved in directional motility.

Because of early studies showing 8-bromo-cGMP stimulation of sperm respiration rates (Hansbrough et al., 1980), that cyclic GMP might represent a "second messenger" for the peptides has remained a viable hypothesis. The analogue studies presented here, however, raise serious doubts that the cyclic GMP concentration, per se, regulates sperm respiration rates. We have yet to address the question of cyclic nucleotide turnover (Goldberg et al., 1983), however, which has been suggested to be of major importance in retina.

ACKNOWLEDGMENTS

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Registry No. cAMP, 60-92-4; cGMP, 7665-99-8; resact, 91316-25-5; [Tyr^1,Ser^8]resact, 101809-74-9; [Ser^1,Tyr^8]resact, 101772-34-3; [Tyr^1,Cys(Me)^8]resact, 101772-35-4; [Cys(Me)^1,Tyr^8]resact, 101772-36-5; [Cys(Me)^1,Cys(Me)^8]resact, 101772-37-6; resact-(1–7), 101772-38-7; resact-(8–14), 101979-13-9; Gly-Gly-Gly-[Tyr^1,Ser^8]resact, 101979-14-0.

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Structural Homologies in the Lutropin/Human Choriogonadotropin Receptor and the Follitropin Receptor on Porcine Granulosa Cells†

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ABSTRACT: In order to examine the structure of the human choriogonadotropin (hCG) receptor and the follitropin (FSH) receptor on porcine granulosa cells, the hormone receptors were photoaffinity-labeled or affinity-cross-linked. The resulting hormone–receptor complexes were analyzed by alkaline cleavage of cross-links, reduction of disulfides, and peptide maps. The results revealed striking similarities in the structure of the hormone receptors. Both appear to be oligomeric; the hCG receptor has at least four components of 18, 24, 28, and 34 kDa, whereas the FSH receptor shows three distinct components of 18, 22, and 34 kDa. The 24- and the 22-kDa components are the sites for the primary photoaffinity labeling or affinity cross-linking by hCG and FSH, respectively. These components were linked by intercomponent disulfides. Reduction of cross-linked complexes revealed that in the hCG receptor the 24-, the 28-, and the 34-kDa components were disulfide-linked sequentially in a linear form as were the 22-, the 18-, and the 34-kDa components in the FSH receptor. The peptide maps of cross-linked hCG–receptor and FSH–receptor complexes, however, were distinct, indicating that the hCG receptor and the FSH receptor were not identical.

Follitropin (FSH)[1] and lutropin (LH) are pituitary glycoprotein hormones and have distinct receptors on ovarian and testicular cells. The placental glycoprotein hormone hCG binds to the LH receptor and elicits similar physiological responses (Ward, 1978; Pierce & Parson, 1981). The structure of these three hormones are quite similar. They consist of two noncovalently associated, dissimilar subunits designated α and β. Within the same mammalian species, the α subunits of these hormones have virtually identical amino acid sequences.

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[1] Abbreviations: FSH, follitropin; LH, lutropin; hCG, human choriogonadotropin; TSH, thyrotropin; PBS, 0.15 M sodium chloride and 10 mM sodium phosphate (pH 7.4); NHS-ABG, *N*-hydroxysuccinimide ester of (4-azidobenzoyl)glycine; ABG-^{125}I-FSH, (4-azidobenzoyl)-glycyl-substituted ^{125}I-labeled follitropin; ABG-^{125}I-hCG, (4-azidobenzoyl)glycyl-substituted ^{125}I-labeled human choriogonadotropin; SES, bis[2-[[(succinimidooxy)carbonyl]oxy]ethyl] sulfone; NEM, *N*-ethylmaleimide; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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