Oxytocin: Effects of Degradation on Radioimmunologic and Biologic Activity

Abstract. *Incubation with thioglycollate destroyed biologic activity of oxytocin, but left immunologic activity intact. Incubation with plasma of pregnant women at term or with placental extract destroyed biologic and immunologic activities. Dissociation of biologic from immunologic sites is suggested.*

A radioimmunoassay for oxytocin (*1*) permits detailed immunological studies of this hormone. We now report a comparison of the biological and immunological activities of oxytocin that was treated with several inactivating agents.

Synthetic oxytocin (Sandoz, 10 μg/ml) was incubated with three inactivators: (i) 0.1*M* sodium thioglycollate, at room temperature for 32 hours; (ii) plasma obtained from pregnant women at term, at 37°C for 12 hours; (iii) an aqueous extract of human placenta, at 37°C for 12 hours. Untreated oxytocin and oxytocin incubated at 37°C for 12 hours served as controls. After incubation with each of these three solutions, oxytocin was determined by radioimmunoassay (*1*) and by biological assay with mammary gland strips from lactating rats (*2*). During a duplicate experiment all incubation periods were 20 hours, and the immunologic activity was tested with a different antiserum.

The immunological activity of the oxytocin was not affected by prior treatment with thioglycollate (Fig. 1), but its affinity for the antibody was decreased and qualitatively altered by treatment with plasma from pregnant women and was virtually destroyed by human placental extract. Bioassay with portions from the same incubation mixtures showed less biological activity with all three inactivators (Table 1).

The data demonstrate dissociation of biologic and immunologic sites on the oxytocin molecule. Similar dissociation has been described for other hormones and has been worked out perhaps in greatest detail for adrenocorticotropic hormone (*3*). Demonstration of such dissociation for oxytocin is of particular interest because oxytocin is composed of only eight amino acids. In so small a molecule, significant overlap of the two sites might have been anticipated. Significant dissociation of immunologic from biologic activity for several analogs of oxytocin and vasopressin has previously been demonstrated (see *1*).

Table 1. Time in seconds (plus or minus standard error of mean of three) between exposure of mammary tissue to oxytocin and the milk-ejection response; OT, synthetic oxytocin (Sandoz); concentration expressed as international units per milliliter; n.c., no contraction in 3 minutes.

Substance	Elapsed time between exposure and response (sec)	
	Experiment 1	
	Oxytocin concentration	
	0.5×10^{-2}	0.5×10^{-4}
OT	27 ± 1.4	34 ± 2.4
OT + thioglycollate	63 ± 11.9	118 ± 20.8
OT + pregnancy plasma*	53 ± 3.6	55 ± 7.1†
OT + placental extract	n.c.	n.c.
	Experiment 2	
	Oxytocin concentration	
	1.0×10^{-2}	1.0×10^{-4}
OT	17 ± 1.4	20 ± 1.4
OT incubated at 37°C	21 ± 1.9	17 ± 2.5
OT + thioglycollate	57 ± 8.3	81 ± 10.7
OT + pregnancy plasma*	53 ± 4.9	97 ± 5.9
OT + placental extract	86 ± 16.6	99 ± 7.1

* Plasma obtained from pregnant women at term. † Mean of duplicates.

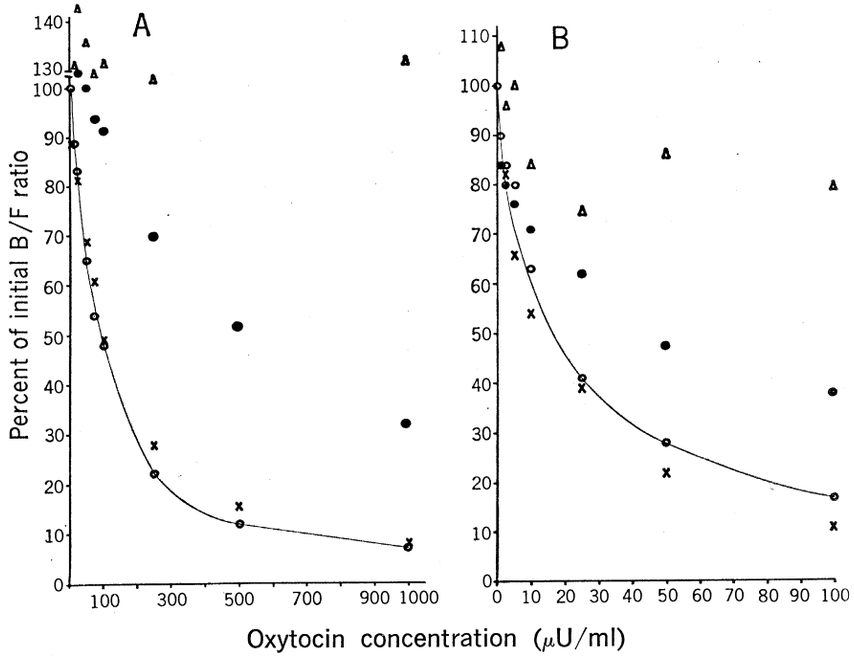

Fig. 1. Reduction of ratio of antibody-bound (B) to free (F) oxytocin-I^{131} as a function of concentration of oxytocin in incubation mixture. Two separate experiments are depicted. (A) Immunoassay results with the materials from experiment 1 (Table 1). Open circles, oxytocin; crosses, oxytocin incubated with thioglycollate; solid circles, oxytocin incubated with plasma from pregnant women; triangles, oxytocin incubated with placental extract. Assay performed with antiserum from rabbit F at 1:10,000 dilution (B) Immunoassay results with the materials from experiment 2 (Table 1). Symbols same as in (A). Assay performed with antiserum from rabbit F-10 at 1:50,000 dilution.

Since 1942 (*4*) thioglycollate has been used to inactivate vasopressin and oxytocin. Its presumed mechanism of action has been the rupture of the S–S bond, with breakage of the ring structure and major alteration of the three-dimensional conformation of the hormones. If this mechanism is the correct one, our data suggest that the disulfide bond, presumably ruptured by thioglycollate, is not part of the immunologically active site, although it may be part of the biologically active site. However, more recent data indicate that thioglycollate cannot act merely by rupturing the disulfide bond, because the disulfide bond is not essential for biologic oxytocic activity (*5*). Nevertheless, whatever the presumed mechanism of action of thioglycollate on oxytocin, it would seem not to affect immunoreactivity, at least for the particular antiserums tested. Because of the variability of antibody formation between individual animals, we have tested antiserums from two animals and have obtained the same results. Conceivably, other immunized animals might produce antibodies which would not react well with thioglycollate-treated oxytocin.

The "oxytocinase" in the circulating

plasma during pregnancy is believed to be an aminopeptidase acting on the hemicystinyl-tyrosyl bond (*6*), but also capable of acting as a relatively unselective aminopeptidase (*7*). Although the placenta is believed to be the source of the oxytocinase in the plasma of pregnant women, it also contains "tissue oxytocinase," which appears first to cleave the disulfide bond of oxytocin, after which degradation by aminopeptidase takes place (*8*). Both oxytocinases destroy biological activity of oxytocin under the conditions described. The greater effectiveness of tissue oxytocinase over that of plasma oxytocinase in destroying immunologic activity may be due to the production of smaller peptides by tissue oxytocinase as a result of the dual action of disulfide cleavage plus aminopeptidase degradation.

Our investigations are of practical importance in immunoassays. It is often essential to assay plasma or body fluids containing no hormone as control materials with which to detect artifactually elevated hormone concentrations in the particular assay system. Our data suggest that extreme caution must be exercised in using proven biologic inactivators as hormonal inactivators in an immunoassay system. Each inactivator must be evaluated for effectiveness in the particular test system employed, be it biological or immunological. The immunoassay of oxytocin might at times measure degraded and biologically inactive fragments of oxytocin.

P. KUMARESAN
A. KAGAN
S. M. GLICK

Coney Island Hospital, Brooklyn, New York 11235

References and Notes

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Group A Streptococci: Localization in Rabbits and Guinea Pigs Following Tissue Injury

Abstract. *Rabbits injected intravenously with extracellular products ("toxins") of group A streptococci develop myocardial, muscular, and hepatic lesions. When such animals are then challenged with fluorochrome-labeled group A streptococci or with titanium oxide particles the labeled bacteria or particles localize within phagocytic cells in the tissue lesions caused by the toxins. Similarly, labeled streptococci or titanium oxide particles will also localize within phagocytic cells in skin lesions of guinea pigs that develop delayed hypersensitivity to tuberculin or to bovine gamma globulin. It is proposed that a combined mechanism of injury and localization of bacteria in damaged tissues may be responsible for post-streptococcal sequelae or other chronic inflammatory diseases.*

In a previous study (*1*) it was shown that a single intramyocardial injection of group A streptococci in rabbits induced granulomatous lesions at the site of injection. Neither trauma to the heart with a needle nor the intravenous injection of streptococci alone caused any lesions in the heart. On the other hand, granulomatous lesions developed in the heart at the site of needle trauma when living or dead streptococci were injected intravenously 24 hours after heart puncture. The experiments suggested that mechanical trauma predisposed to the localization of streptococci that could induce a granulomatous response. In addition, extracellular products (SEP) of group A streptococci induced severe myocardial, hepatic, and diaphragmatic lesions in rabbits after intravenous injection (*2, 3*).

This production by a group A streptococcus of a "tissue-damaging toxin" raised the possibility that, by analogy to "needle trauma," such toxic components may prepare the heart for the localization of streptococci. In this report we propose a possible mechanism by which group A streptococci and some of their components localize in tissues of rabbits injured by SEP or by unrelated delayed hypersensitivity reactions.

Myocardial, diaphragmatic, and hepatic lesions were induced in New Zealand white rabbits, weighing 1.5 to 2.0 kg, by the intravenous injection of SEP as described previously (*2, 3*). A fraction containing the tissue-damaging factor (TF) was isolated from SEP by gel filtration through Sephadex G-150 columns and was found to be associated with the high molecular weight material excluded from the column. The TF contained two antigens that reacted with antiserum to SEP and two protein components as revealed by gel electrophoresis. This fraction did not contain any detectable amounts of hyaluronidase, nicotinamide adenine dinucleotide nucleosidase activity, streptokinase, C-polysaccharide, or M-protein, but contained trace amounts of deoxyribonuclease and substantial amounts of streptolysin O, acid phosphatase (*4*), and a cell-sensitizing factor (*5*).

When injected intravenously into rabbits, 1 to 2 mg of TF protein (*6*) regularly caused severe coagulation necrosis in the liver, interstitial myocarditis, and a steep rise in serum levels of glutamic oxaloacetic transaminase and total lipids (*3*). Some of the animals were then injected intravenously or intraperitoneally with 1 ml (10^8 cell/ml) of washed type 4 streptococci that had been labeled directly with fluorescein isothiocyanate (FITC) or with rhodamine isothiocyanate (RITC) according to a method described previously (*7*). Other animals treated with TF were injected with titanium oxide (TiO_2) particles (100 per kilogram of body weight) as a control for nonspecific uptake of particles by the reticuloendothelial system (*8*).

Delayed hypersensitivity was induced in Hartley guinea pigs weighing 300 to 350 g by immunization (footpad injection) with 100 μg of bovine gamma globulin (BGG) in complete Freund's adjuvant that contained 2 mg of *Mycobacterium tuberculosis* (strain H37RV). Thirteen days after the injection of BGG, the animals received an intravenous injection of FITC- or RITC-labeled type 4 streptococci or of TiO_2 as described above. Four hours later several of the animals were challenged intracutaneously with PPD (purified protein derivative) (20 μg per injection site) while others received BGG (40 μg per injection site). A second injection of labeled streptococci or of TiO_2 was given intraperitoneally 8 hours after injection of the antigens. Twenty-four hours after intracutaneous injection of antigen, the animals developed typical delayed hypersensitivity lesions in the skin.

All animals were killed 1 to 7 days