Inactivation of Prolactin by Human Blood Serum and Plasmin*† (Fibrinolysin). (26263)

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Inactivation of several protein hormones by various biological tissues has been reported. Adrenocotropin (ACTH) is readily inactivated when incubated with whole blood, blood plasma(1-3), or fibrinolysin(4); glucagon, ACTH and somatotropin are inactivated by streptokinase-activated blood plasma or plasminogen(5,6); and insulin, ACTH and prolactin activities are reduced by various mammalian tissues and tissue homogenates (7-10). The present report deals with the effects on prolactin activity of incubation with human blood serum and fibrinolysin (derived from human blood).

Methods. Purified ovine prolactin‡ with an activity of 15 I. U. per mg was used in these studies. The prolactin was dissolved in distilled water to a concentration of 5.0 mg prolactin per ml solution; 0.1 ml (0.5 mg) prolactin solution was added to each flask which contained 0.9 ml incubation medium. Serum concentrations were prepared by diluting pooled serum, representing 6 different blood samples, with Krebs-Ringer phosphate buffer of pH 7.2. Three consecutive assays were performed employing pooled serum which was aliquoted, stored in a freezer and thawed just before use. Plasmin§ solutions were prepared by adding 0.1 ml (5.7 caseinolytic units; 14.1 Loomis units) fibrinolysin solution to 0.8 ml Krebs-Ringer phosphate buffer. Total volume of each incubation sample was 1.0 ml. As a control, standard prolactin was added to Krebs-Ringer phosphate buffer and incubated simultaneously with the serum and fibrinolysin preparations. Following 2 hours incubation at 37°C, each sample was diluted 10-fold with saline solution to give an initial prolactin concentration of 0.05 mg/ml.

Prolactin activity was assayed in White Carneau pigeons employing the method of Reece and Turner(11). Each pigeon was given 0.1 ml assay solution daily for 4 days. On the fifth day the pigeons were sacrificed and the crop sacs were removed and rated. By injecting the incubated standard prolactin preparation over one crop sac and comparing the response with that of an equivalent amount of experimental sample injected over the other crop sac, each bird served as its own control. To minimize subjectivity, ratings were made by 2 independent coworkers who were unfamiliar with the assay materials but experienced with the assay technic.

Results. Incubation of prolactin with human blood serum and with fibrinolysin caused significant losses in prolactin activity (Tables I and II). Incubation of 0.5 mg prolactin in 10% serum (Table I) resulted in moderate but significant losses (33, 36 and 40%) of prolactin activity. The activity of a similar amount of prolactin incubated in 45% serum produced a mean pigeon crop re-

TABLE I. Mean Loss of Prolactin Activity Following Incubation in Human Blood Serum. (0.5 mg prolactin incubated in each exp.)

No. pigeons used per assay	Avg rating of pigeon crops		Loss of prolactin activity	
	Std. prolactin	Incubated in 10% serum	mg	%
4	2.56	1.50	.20	40
4	1.56	1.00	.18	36
4	1.40	.95	.17	33
		Avg	.18	36 ±2.0*
		Incubated in 45% serum		
3	1.91	.58	.35	70
5	1.70	1.00	.21	41
3	2.42	1.42	.26	52
		Avg	.27	54 ±8.5*

* Stand. error.

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TABLE II. Mean Loss of Prolactin Activity Following Incubation with Fibrinolysin (5.7 cu). (0.5 mg prolactin incubated in each exp.)

No. pigeons used per assay	Avg rating of pigeon crops		Loss of prolactin activity	
	Std. prolactin	Incub. with fibrinolysin	mg	%
7	1.39	.21	.43	85
7	1.57	.07	.48	96
10	1.37	.0	.50	100
		Avg	.47	94±5.2*

* Stand. error.

sponse that was only 46% of the standard response, indicating a 54% loss of prolactin activity. Incubation with fibrinolysin resulted in essentially a complete loss (85, 96 and 100%) of prolactin activity (Table II).

Discussion. These data show that human blood serum and human fibrinolysin can inactivate ovine prolactin. The factor(s) in human blood serum which inactivate prolactin is unknown; however, it is possible that the fibrinolysin system is at least partly responsible for the loss of activity. Previously it was demonstrated that prolactin was inactivated by several other tissues, including mammary gland, ovaries, liver and kidney of rats and pigeon crop glands(9,10). Similarly it has been shown that ACTH is inactivated when incubated with rat liver and kidney slices, diaphragm, and homogenates of liver, kidney and adrenal tissues(8). The inactivating systems of these tissues were not described.

Fibrinolysin is a proteolytic enzyme, found in all mammalian blood sera, but normally present as the inactive precursor, plasminogen(12). Addition to blood plasma of factors known to activate plasminogen has increased the hormone-inactivating efficiency of human plasma(6). Recently it has been shown that a "factor" extracted from human blood plasma will activate (a) plasminogen and (b) blood plasma fractions in which plasminogen is concentrated (Hopkins, Terner and Pincus, unpublished). Both activated products readily inactivated ACTH.

Since prolactin was inactivated by human blood serum (and more recently by bovine, rabbit and rat sera, unpublished) without addition of known plasminogen activators, we investigated the presence in prolactin of plasminogen-activator contaminants. In the fibrin plate test, prolactin activated human plasminogen, causing lysis of fibrin.‖ The presence of plasminogen activators has previously been demonstrated in human pituitary tissue(12). Inactivation of prolactin by human serum and activation of plasminogen by prolactin cannot be interpreted at this time to indicate that the fibrinolysin system alone is responsible for loss of prolactin activity.

Summary. Ovine prolactin (7.5 I.U.) was incubated for 2 hours at 37°C with human blood serum and human fibrinolysin. Moderate decreases of prolactin activity occurred after incubation in 10% serum, and somewhat greater losses occurred when incubated in 45% serum. Fibrinolysin caused almost complete inactivation of prolactin. The relationship of the serum inactivator system to the fibrinolysin system remains to be established.

‖ Performed by Dr. J. T. Sgouris, Mich. State Health Labs.

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