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Comparison of Systemic and Uterine Effects of Relaxin and Insulin in Alloxan-Treated, Hyperglycemic Rats (41360)

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Abstract. The effects of porcine relaxin and insulin on blood glucose and on uterine and diaphragm glycogen were measured after 3 and 24 hr in alloxan-treated, hyperglycemic rats. Insulin caused a decrease in blood glucose and enhanced the glycogen concentration of diaphragm muscle but had no effect upon the uterus. Relaxin, which had no influence on blood glucose levels, increased uterine weight and uterine glycogen content; diaphragm glycogen concentration was depressed at 3 hr after relaxin administration. Neither insulin nor relaxin altered the action of the other hormone when given concurrently. Despite the structural similarities between insulin and relaxin, the two hormones exhibit distinctly different actions which were not found to overlap.

The structure of relaxin reported by Schwabe and McDonald (1) bears considerable homology to that of insulin. Relaxin has been shown to be identical to insulin with respect to its disulfide bond distribution and disulfide ring size, and 18 of the 48 residues are identical to or conservative replacements of corresponding amino acids in insulin. Space filling models and circular dichroism studies have affirmed the structural homology of the two hormones (2–5). Because of these similarities relaxin is often classified with the family of insulin-like growth factors, including nerve-growth factor, somatomedins, and insulin, with both structural and functional similarities in modulating cell growth and activity (6).

Despite its similarity to insulin, relaxin is not known to possess systemic insulin activity, nor does it cross react immunologically with insulin or compete with insulin for receptors (4, 5). Relaxin binds to various reproductive tissues but not to liver and heart, tissues to which insulin characteristically binds (7). The similarity between the two hormones, however, has led to the suggestion that relaxin may influence the reproductive tract in a manner similar to the systemic regulation of metabolism and growth by insulin (8). Relaxin has been shown to increase uterine weight, protein, and glycogen in ovariectomized immature rats (8–10) and in cycling and pregnant rats (11). While these experiments have shown that relaxin has protein and carbohydrate anabolic effects in the uterus, relaxin did not affect diaphragm glycogen concentrations or blood glucose levels (8).

It is in hyperglycemic animals that the most striking effects of insulin are seen. Therefore, it is of interest to determine whether relaxin exhibits systemic insulin-like effects under these conditions or whether insulin affects the reproductive tract when blood glucose is abnormally high. We report here the results of experiments undertaken in order to compare the actions of insulin and relaxin, both systemically and in the uterus, in alloxan-treated, hyperglycemic rats.

Materials and Methods. Sprague–Dawley rats were bilaterally ovariectomized at 30 days of age and injected ip with alloxan (125 mg/kg) 6 days later. After one day the animals received 5 μg estradiol benzoate sc. Seven days following estrogen priming the animals were injected sc with either saline (0.2 ml), relaxin[3] (0.1 mg in 0.2 ml saline), insulin[4] (0.1 mg in 0.2 ml saline),

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[3] Purification of porcine relaxin yields three electrophoretically distinct peptides with relaxin activity. The peptide used in these experiments was relaxin B which appears to be identical with relaxin CM-B (22) and the preparation sequenced by Schwabe and McDonald (1).

[4] Bovine insulin (23.6 IU/mg) was purchased from the Sigma Chemical Company.

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or insulin plus relaxin (0.1 mg in 0.2 ml saline, each) and killed 3 hr later (Experiment I). In the second experiment, rats were injected with either 0.2 ml of 1% benzopurpurine 4B (BP), relaxin (0.05 mg in 0.2 ml BP), insulin (0.05 mg in 0.2 ml BP), or insulin plus relaxin (0.05 mg in 0.2 ml BP, each) at 24 and 12 hr prior to sacrifice. Benzopurpurine was chosen as the vehicle for the hormones in the 24-hr experiment because of its ability to potentiate the action of relaxin (9); its effect on insulin action is unknown. Blood samples (0.1–0.2 ml) were taken by cardiac puncture, under light ether anesthesia, at the time of the first injection. Rats were killed by cervical dislocation; blood was collected and uteri and diaphragms were excised and weighed. Blood glucose and uterine and diaphragm glycogen were determined by methods previously described (8). At the time of injection of relaxin or insulin only those animals were used which had blood glucose levels in excess of 150 mg/100 ml, twice the normal level of fasting blood glucose in our colony animals (8).

Means of all data were compared by Student's *t* test and statistical significance reported with a probability level of 0.01 or 0.05, as noted.

Results. Three hours after relaxin administration (0.1 mg) to alloxan-treated rats, blood glucose concentration did not decrease and in some rats a sharp rise was noted although the data varied considerably and did not differ significantly from vehicle-injected controls or from the initial values (Table I). Insulin (0.1 mg), however, significantly reduced blood glucose over this time period with or without concomitant relaxin injection (Table I). After 3 hr uterine weight was increased (40%) in relaxin-treated rats, while insulin alone had no effect (Fig. 1). Uterine glycogen concentration was not altered significantly in any group, although relaxin produced a significant decline in diaphragm glycogen (50%) and insulin increased this component. When relaxin and insulin were given together the increase in diaphragm glycogen was no different from that of animals receiving insulin alone, and the uterine changes were comparable to those in tissues from rats given relaxin only.

Twenty-four hours after the first relaxin injection the uterine weight increase in alloxanized rats was no longer significant although uterine glycogen content was some 60% higher than controls (Fig. 1). No change was apparent in blood glucose or diaphragm glycogen concentration. Insulin (in BP) had no effect on uterine weight or uterine glycogen concentration but increased diaphragm glycogen concentration by 70% (Fig. 1) and decreased blood glucose (40%) 24 hr after treatment (Table I). There was no interaction between the effects of relaxin and insulin on tissue glycogen after 24 hr but in this latter case the residual blood glucose depression did not differ significantly from untreated controls.

Discussion. The hypoglycemic effects of

TABLE I. BODY WEIGHTS AND BLOOD GLUCOSE CONCENTRATIONS IN ALLOXAN-TREATED, HYPERGLYCEMIC RATS

	Experiment I			Experiment II		
	Body weight (g)	Blood glucose (mg/100 ml)		Body weight (g)	Blood glucose (mg/100 ml)	
		Initial	3 hr		Initial	24 hr
Control	165 ± 7	270 ± 61	286 ± 59	120 ± 6	406 ± 40	318 ± 60
Relaxin (0.1 mg)[a]	144 ± 15	249 ± 51	331 ± 81	140 ± 10	441 ± 48	407 ± 56
Insulin (0.1 mg)[a]	132 ± 15	320 ± 65	93 ± 25	134 ± 10	441 ± 41	260 ± 40
Relaxin + insulin	151 ± 14	224 ± 46	63 ± 29	151 ± 8	341 ± 72	258 ± 63

[a] In Experiment I, hormones were administered as a single dose in 0.2 ml saline; in Experiment II the doses were divided (0.05 mg each) and administered at 24 and 12 hr preceding sacrifice and benzopurpurine 4B was used as the vehicle. Controls received the appropriate vehicle in each experiment.

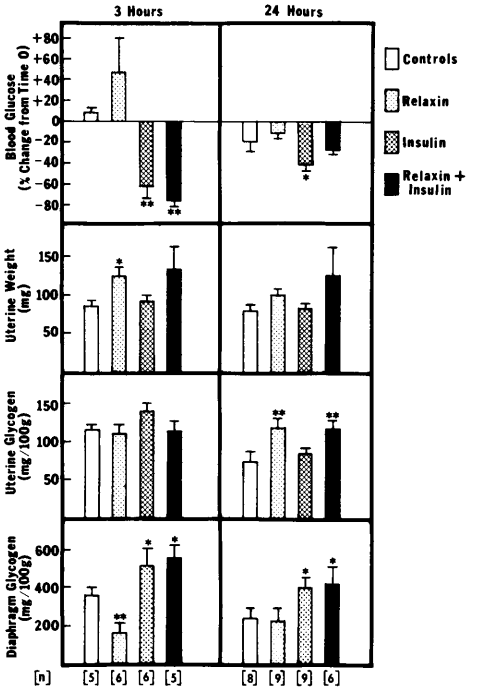

FIG. 1. The effects of relaxin and insulin in ovariectomized, alloxan-treated, estrogen-primed rats. All controls received vehicle alone, sc. In the 3-hr experiment (I), 0.1 mg of relaxin, insulin, or both hormones were injected in saline in a single dose, while for the 24-hr experiment a total of 0.1 mg of each hormone was injected as a suspension in 1% benzopurpurine 4B in two doses (0.05 mg each) at 24 and 12 hr prior to sacrifice. Vertical lines represent standard errors of the means. Significant differences from controls are indicated by * and ** which represent $P < 0.05$ and 0.01, respectively.

insulin are reported to be maximal between 1 and 6 hr postinjection (12) and in our experiments a single injection of insulin (0.1 mg) produced the expected decrease in blood glucose and increase in diaphragm glycogen concentration with no effect on the uterus, similarly to previous reports (13, 14).

Alloxan itself, by virtue of its hyperglycemic effect, has been shown to increase glycogen concentration after a minimum of 48 hr following treatment (15), although estrogen treatment minimized differences in uterine glycogen between normal and alloxan-diabetic rats (14). The prolonged effects of insulin on blood glucose and diaphragm glycogen concentration after 24 hr may be attributable to the potentiation of insulin effects in hyperglycemic animals by the vehicle benzopurpurine. In contrast to insulin, relaxin increased uterine weight and glycogen content. The time sequence of the uterotrophic and glycogenic effects of relaxin are in agreement with previous reports (8, 9); using the 24 hr, two-dose regime, the uterotrophic effect of relaxin is observed within the first 6 hr while the glycogenic effects are maximal 12 hr after treatment. Furthermore, neither insulin nor relaxin augmented the action of the other hormone when given concurrently. The two hormones appear to exhibit separate effects in alloxan-treated, hyperglycemic rats each independently of the action of the other and regardless of blood glucose levels.

Although no systematic study has been conducted on the role of various structural features of relaxin in its activity, there is evidence that the C-terminal region of the B chain is not important for biological activity (16). This is in contrast to insulin, where there is evidence that B_{22-26} of the C-terminal end is the active site (17). This region of insulin has been known for some time to be of crucial importance for its biological actions and is nearly invariant among many animal insulins (18). The substitution of B_{23} glycine by an L-amino acid has been shown to abolish insulin's biological activity (19). Another relevant structural difference occurs in the A chain. When A_{21} asparagine is removed from insulin so that, like relaxin, its A chain ends at A_{20} cystine, the hormone's structural properties are altered, and its biological potency is much reduced (20, 21). In diverging from insulin at A_{21} and B_{23}, relaxin should therefore be without the capacity to mimic insulin (3), and our data support that prediction.

While insulin controls blood glucose levels and anabolic metabolism in various tissues, it does not influence glycogen or protein metabolism in the uterus. On the other hand, the anabolic effects of relaxin appear to be confined to the reproductive tract. Thus, despite the structural simi-

larities between insulin and relaxin, the two hormones exhibit distinctly different actions which have not been found to overlap in either the normal or the hyperglycemic rat, although it is possible that relaxin exerts effects on blood glucose and diaphragm glycogen in a manner opposite to that of insulin in the latter condition. It is also conceivable that the two hormones may yet be found to act on their respective target tissues by means of similar cellular mechanisms.

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