A Comparison of the Effects of Thyrocalcitonin and Glucagon on Plasma Calcium and Phosphate[1] (34506)

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Liver glycogenolysis and the resultant temporary hyperglycemia which follows the administration of glucagon have been well documented in both experimental animals and man. Recently, interest has been generated in studies of extrahepatic effects of glucagon, particularly in regard to its possible influence on calcium and phosphate homeostasis. As early as 1955, DeVenanzi (1) reported a hypophosphatemia after glucagon administration, a result which Staub (2) later attributed to the renal effects of the hormone.

Several investigators (3–5) have reported a transient hypocalcemia after glucagon administration in several mammalian species, including man, and Avioli (6) concluded that the hormone was a releasing factor for thyrocalcitonin from the thyroid. Paloyan (3) has suggested an interreaction between glucagon, parathyroid hormone, and thryocalcitonin at the level of renal function. However, a direct effect of glucagon on renal calcium excretion has been ruled out as the primary cause of hypocalcemia by studies in nephrectomized rats and dogs (4, 6). Stern and Bell (7) postulated that glucagon acted directly on bone metabolism in a manner similar to thyrocalcitonin by directly inhibiting parathyroid-stimulated bone resportion.

Though hypophosphatemia is known to result from glucagon or thyrocalcitonin administration, the effects of these agents on phosphate homeostasis have escaped serious attention. Talmage and colleagues (8–10) have proposed a direct relation of thyrocalcitonin to intracellular phosphate metabolism in bone, and have suggested that the calcemia effects of the hormone may be secondary to its effects on phosphate.

The purpose of the study to be reported here is to examine more closely the interrelationship of these three effects; *i.e.*, hypophosphatemia, hypocalcemia, and liver glycogenolysis of glucagon, and to compare the action of this hormone with that of thyrocalcitonin, with particular emphasis on the relation of both of these hormones to phosphate homeostasis.

Materials and Methods. Male Holtzman and Cheek-Jones rats, weighing between 180 and 200 g were used in these experiments. To minimize variations in starting liver glycogen concentrations, the animals were fed a stock diet during a restricted time period between 5:00 and 10:00 PM daily. On the evening prior to hormone administration, the feeding period was terminated at 7:30 PM, and deionized water replaced the tap water provided for the animals.

Treatment of the rats prior to hormonal administration included the following as needed: (1) radiophosphorus (^{32}P) administration either 10 days or 18 hr prior to use; (2) parathyroid transplantation as previously described (8); (3) thyroidectomy of animals with functional parathyroid transplants at least 48 hr before use, followed by daily administration of thyroxin (25 μg/kg body wt/day); (4) surgical parathyroidectomy at 11:00 PM the night prior to hormone injection; and (5) bilateral nephrectomy at times indicated in the particular experiments. All hormone injections were given between 9:00 and 10:00 A.M. Blood samples were obtained from the tail, limiting each bleeding to approximately 100 μl.

The glucagon used was a crystalline prepa-

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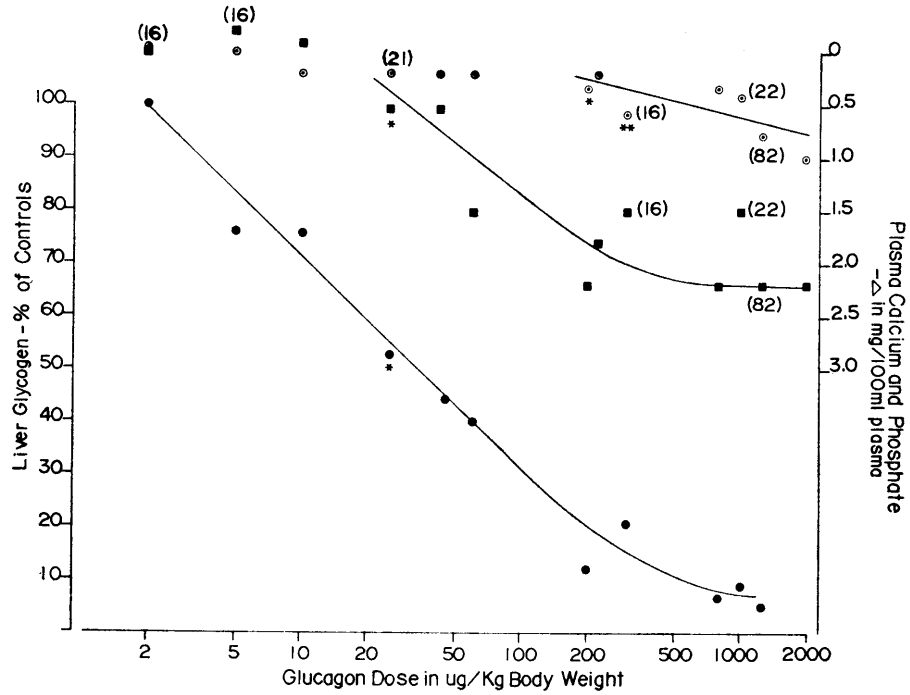

FIG. 1. Dose-response curves after glucagon administration. Plasma calcium and phosphate values expressed as change from controls which are indicated by shaded area. *values for these and larger doses are significant from control values at $p < .05$. **values for these and larger doses are significant from control values at $p < .001$. All points except those shown in parenthesis, represent 6-10 animals. ● = liver glycogen. ■ = plasma phosphate. ⊙ = plasma calcium.

ration,[4] and rat thyrocalcitonin was prepared as previously described (9). Shark calcitonin was provided through the courtesy of D.H. Copp.

Plasma calcium was analyzed by the fluorometric procedure of Kepner and Hercules (11), and plasma phosphate according to Chen and Toribara (12). Liver glycogen was extracted and hydrolyzed, and glucose was determined by the anthrone method (13). Radioactivity was measured by means of a liquid scintillation system with 20 μl of plasma dissolved in a fluor consisting of 4 g PPO, 0.2 g dimethyl POPOP, and 60 ml of Beckman biosolve (BBS-3) per liter of toluene. Standard errors were calculated for all experimental groups, and all data were subjected to Student's *t* test for statistical significance.

Results. Dose-response curves after glucagon administration. Glucagon was injected subcutaneously into normal rats in doses ranging from 2 to 2000 μg/kg body wt. A dose-response curve was determined for liver glycogen depletion and for hypophosphatemic and hypocalcemic effects. Blood samples were taken prior to glucagon injection and 1 hr postinjection. The animals were immediately sacrificed by cervical dislocation and samples from the same location in the liver were obtained for glycogen analysis. Because of the necessity to obtain liver glycogen values, only 1 hr postinjection plasma values could be obtained. The results are summarized in Fig. 1.

The liver glycogen depletion curve is consistent with known carbohydrate effects of glucagon. It appears that glycogenolysis occurs with a dose as small as 5 μg/kg body wt. A near-maximal response is achieved with a dose of 1000 μg/kg. Since the rats were not able to serve as their own controls, the variability in data is rather large, delaying a sta-

[4] Eli Lilly crystalline glycagon.

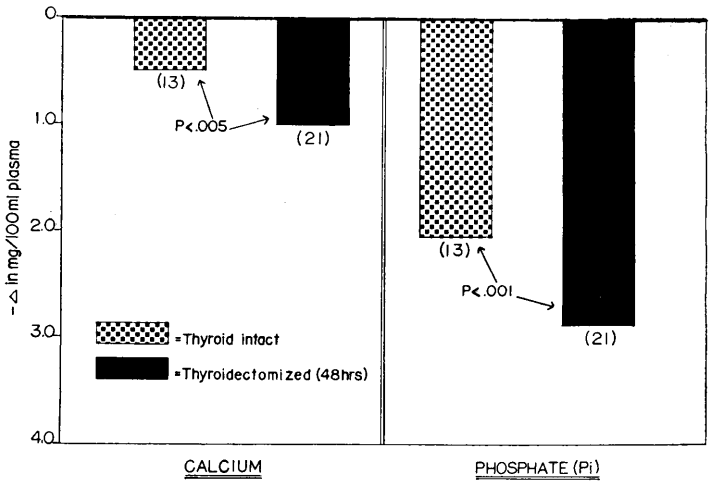

FIG. 2. Comparison of the hypocalcemic and hypophosphatemic effects of glucagon in intact and thyroidectomized animals. Numbers in parentheses = number of animals. Plasma values 1 hr after injection of 1.25 mg glucagon/kg body wt.

tistically significant drop in liver glycogen until a dose of 45 μg/kg was given.

The hypophosphatemic response appeared next, becoming apparent at a dose of less than 20 μg of glucagon/kg. While each rat served as its own control, plasma phosphate values in experimental animals were also compared to those in noninjected controls. The first statistically significant change was with a glucagon dose of 25 μg/kg body wt. The response appeared to level off at the same dose as the glycogenolytic response.

The hypocalcemic response was the last to appear, with a significant reduction in plasma calcium not occurring until a glucagon dose of 250 μg/kg was given. At a dose of 2 μg/kg a drop in plasma calcium values of 1 mg/ml had occurred by 1 hr postinjection.

In corollary studies, rat thyrocalcitonin and shark calcitonin, at doses which produce hypocalcemic responses of 3 mg/100 ml plasma, had no glycogenolytic effect.

The effect of glucagon injections in thyroidectomized rats. All animals in this study bore functional homologous parathyroid transplants permitting subsequent thyroidectomy without interference to parathyroid function. Each rat received the same dose of glucagon (1.25 mg/kg). One hour postinjection values were compared to preinjection values and to noninjected controls. The results are summarized in Fig. 2. As indicated in the dose-response curves, the hypophosphatemic effect was much greater than the hypocalcemic. Also, the decreases in plasma values were significantly greater in the thyroidectomized animals than in the thyroid-intact rats. This is similar to the results reported by Talmage and Kennedy (9) for changes after thyrocalcitonin administration to thyroidectomized rats.

Glucagon and thyrocalcitonin administration to nephrectomized rats. For these studies, glucagon, when administered, was injected at a dose of 1.25 mg/kg body wt, and rat thyrocalcitonin extract at a dose equivalent of 25 MRC milliunits. The thyrocalcitonin study was a duplication of that reported by Kennedy *et al.* (10), and it is included as a comparison of the effects of the two hormones. The hormones were administered to the same group of rats four times prior to nephrectomy for control study, and 1, 12 and 24 hr postnephrectomy. The results are summarized in Fig. 3. The following observations can be made: (1) Glucagon duplicates the action of thyrocalcitonin with minor differences in the hypophosphatemic response; (2) Both hormones are effective in the absence of the kidney; and (3) The hypocalcemic effect increases as the preinjection plasma phosphate level rises.

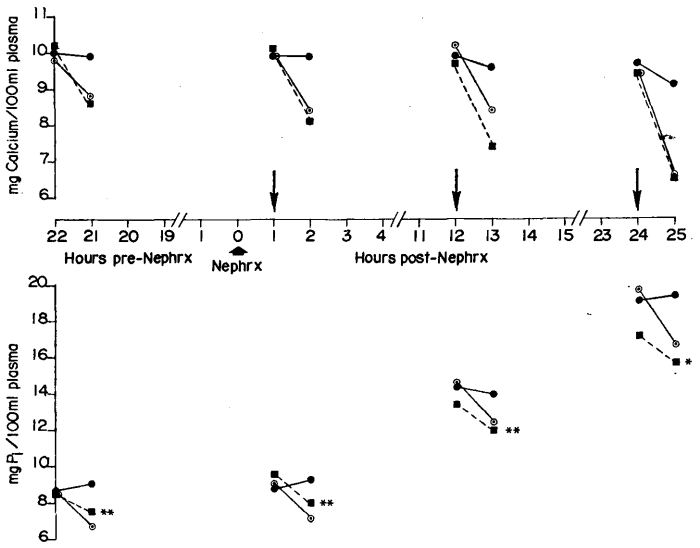

FIG. 3. Comparison of hypocalcemic and hypophosphatemic responses to glucagon and rat thyrocalcitonin in control and nephrectomized rats. Indicates time of injection. All injected animals have significance of $p < .001$ from controls except where indicated. * = difference from preinjected values with $p < .05$. ** = difference from preinjected values with $p < .005$. Each value represents average 12 or more animals. ●-● = control. ⊙-⊙ = glucagon. ■-■ = rat thyrocalcitonin.

Glucagon and thyrocalcitonin administration to parathyroidectomized rats. This series of experiments was similar to that reported in the section above except that an additional group 10 hr after parathyroidectomy was included, and only one time period (1 hr post nephrectomy) was used after nephrectomy because of the failure of the parathyroidectomized rats to survive 24 hr. The results are summarized in Fig. 4. The hormones are obviously effective in parathyroidectomized and parathyroidectomized-nephrectomized rats.

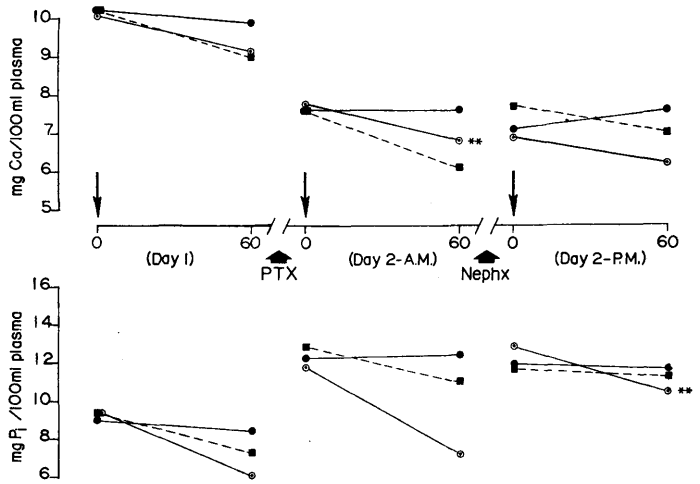

FIG. 4. Comparison of hypocalcemic and hypophosphatemic responses to glucagon and rat thyrocalcitonin in parathyroidectomized and parathyroidectomized-nephrectomized rats. Indicates times of injection. All injected animals are significantly different from control at $p < .001$ except where indicated. ** = difference from preinjected values with $p < .005$. Each value represents average of 12 or more animals. ●-● = control. ⊙-⊙ = glucagon. ■-■ = rat thyrocalcitonin.

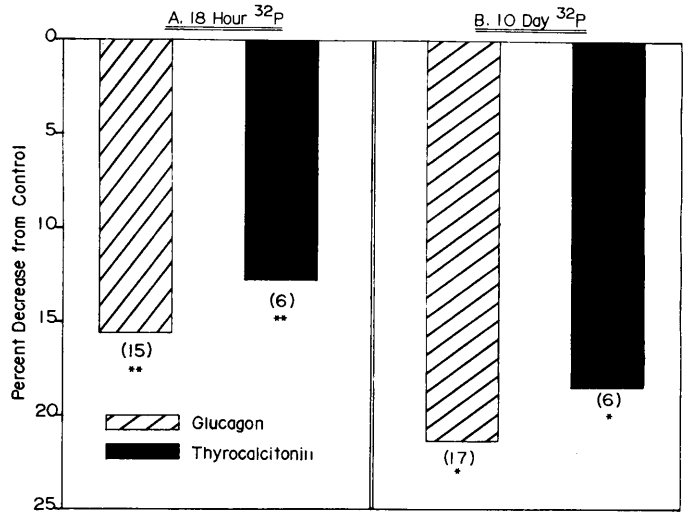

FIG. 5. Plasma ^{32}P changes 1 hr after glucagon administration. A. ^{32}P administration 18 hr prior to glucagon injection. B. ^{32}P administered 10 days prior to glucagon injection. * = $p < .05$ when compared to controls. ** = $p < .001$ when compared to controls.

The observation can again be made that the higher the preinjection plasma phosphate level, the greater the hypocalcemic effect of the two hormones.

Plasma ^{32}P changes after glucagon and thyrocalcitonin injection. Several groups of animals were given radiophosphorus as described above. The glucagon dose was 1.25 mg/kg body wt, and the dose of rat thyrocalcitonin was equivalent to 25 MRC milliunits. Radiophorphorus concentrations in plasma at 1 hr postinjection were compared to the preinjection plasma concentrations. The results are summarized in Fig. 5. Both hormones were effective in reducing plasma radiophosphorus activity in animals in which the ^{32}P had been administered either 18 hr or 10 days prior to hormone injection. The thyrocalcitonin results confirm those of Klein *et al.* (14) and are given here for comparison with glucagon effects.

Discussion. To summarize the findings of these studies, several conclusions seem pertinent:

1. The influence of glucagon on liver glycogen depletion is in line with past work. The use of glycogen depletion rather than hyperglycemia as a test of glucose release from the liver provided for us a more reliable test of hormone action without the interference from subsequent insulin effects.

2. These studies confirm a hypocalcemic effect of glucagon, but at a dose far above the physiological range of the hormone. The important point here is that the hypophosphatemia parallels more closely the hyperglycemic effect and therefore occurs at a much lower dose level than does the hypocalcemic effect.

3. After two or more successive injections of glucagon, resulting in liver glycogen so low as not to be markedly affected by another glucagon administration, the hypocalcemic and hypophosphatemic responses are still elicited, indicating these responses are at least partially independent of the hepatic effect of glucagon.

4. These studies demonstrate for the first time that the effect of glucagon on these electrolytes, particularly calcium, is independent of the thyroid. Since the plasma changes were determined only at 1 hr after glucagon administration, owing to the necessity of sacrificing the animals to obtain liver glycogen values, the experiments do not necessarily disprove a secondary hypocalcemic effect via stimulation of thyrocalcitonin secretion.

5. Likewise, since the hormone was effec-

tive in nephrectomized rats, these actions are independent of renal effects, but do not necessarily rule out such additional effects.

6. Since radioactivity in plasma, for that which had been administered 2 to 3 weeks previously, was suppressed by glucagon, it is suggested that the hypocalcemic and part of the hypophosphatemic effects result from a direct action of glucagon on bone.

And, finally, it is concluded that glucagon at large doses has a thyrocalcitonin-like activity which is probably neither physiological nor related to its primary activity, that of a liver glycogenolytic agent.

Summary. The effect of glucagon has been compared to rat thyrocalcitonin under experimental conditions which had produced information concerning the role of the latter hormone in bone metabolism. The dose-response curve shows that the minimum dose of glucagon needed to produce hypocalcemia produced a dramatic hypophosphatemia and a near-maximum glycogenolytic response. When intact, parathyroidectomized and thyroidectomized rats are treated with pharmacological doses of glucagon, plasma calcium and plasma phosphate changes are similar to those which occur after thyrocalcitonin treatment, thus eliminating the presence of parathyroid and thyroid glands as prerequisite for these effects. Since glucagon is effective in bilaterally nephrectomized animals, these changes can not be due solely to renal effects of the hormone. As with thyrocalcitonin, as the plasma phosphate rises, the hypocalcemic response to glucagon becomes more pronounced. In experiments using either 10-day or 18-hr radiophosphorus labels, glucagon produced a thyrocalcitonin-like action suggesting that, at the doses studied, glucagon may reduce bone resorption. It is concluded that large amounts of glucagon have a thyrocalcitonin-like effect which is probably neither physiological nor related to its primary action as a liver glycogenolytic agent.

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