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Glucocorticoids Possess Calcitonin-Like Antihypercalcemic Properties in Rats

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The interaction among parathyroid hormone (PTH), calcitonin (CT), and glucocorticoids on blood calcium (Ca) was examined. Prior studies had shown that adrenalectomy (ADX) reduced the fall in blood calcium in rats after parathyroidectomy (PTX). Convincing evidence was provided showing that the ADX effect in PTX rats was due to the loss of corticosterone, the major glucocorticoid in rats; restoring physiological blood levels of corticosterone abolished the ADX effect in PTX rats.

The initial attempt of the present study was to explain the failure of ADX or exogenous glucocorticoids to alter serum Ca levels in rats with intact thyroid and parathyroid glands or in thyroidectomized rats with functional parathyroid transplants (PTT). We found, as previously reported, that the 5-h level of serum Ca in rats with parathyroid glands was not affected by sc hydrocortisone (cortisol) or by ADX. It was also not affected by thyroparathyroidectomy (TPTX) or after both ADX and TPTX in rats with PTT.

These results suggested to us that the glucocorticoid effect to lower serum was inhibited by endogenous parathyroid hormone (PTH) from the parathyroid gland and/or by normal levels of blood Ca. Both of these proposed mechanisms were examined and failed to explain the absence of the ADX effect as well as the glucocorticoid effect in normocalcemic parathyroid-intact rats, because an ADX effect was observed in TPTX rats given hypercalcemic doses of rat or bovine PTH 1-34 or calcitriol. Also, administered cortisol restricted the increased hypercalcemia induced by PTH in ADX-TPTX rats. Expanding on the results in TPTX rats with induced hypercalcemia, we found that neither the ADX effect nor the glucocorticoid effect occurred in thyroid-intact rats with or without functional PTT. These as well as previous results show that:

1. Glucocorticoids, like CT, restrict hypercalcemia in TPTX rats.
2. The ADX effect and its reversal by glucocorticoids in rats with induced hypercalcemia occur only in the absence of the thyroid gland (removal of CT).
3. Gucocorticoids, like CT, lower serum calcium during the hypocalcemia after PTX, an effect that occurs in the presence or absence of the thyroid gland.

This study did not reveal why neither ADX nor exogenous glucocorticoids altered serum calcium levels in normocalcemic rats with either intact parathyroid glands or PTT. We conclude that under appropriate conditions, glucocorticoids act in a fashion similar to that of CT in restricting hypercalcemia and in lowering blood Ca.

Key Words: Glucocorticoids; calcitonin-like action; antihypercalcemic effect.

Introduction

There is considerable evidence that glucocorticoids affect calcium (Ca) metabolism, and bone. For example, glucocorticoids are used clinically to lower blood Ca in patients with hypercalcemia *(1,2)*. Also some patients with adrenal insufficiency exhibit hypercalcemia *(3,4),* and corticosteroid-deficient rats have lower bone mass *(5,6)*. Further, the use of supraphysiologic doses of glucocorticoids as anti-inflammatory and immunosuppressive agents in humans leads to bone loss, a condition called glucocorticoid-induced osteoporosis *(7–9)*.

The present study began by examining another effect of glucocorticoids, their Ca-lowering action in rats after parathyroidectomy (PTX). Adrenalectomy (ADX) in rats reduces the fall in blood Ca after PTX *(10–14)*; we have termed this action of ADX, the ADX effect. As shown in our earlier study *(14),* the ADX effect did not occur in PTX

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rats if corticosterone, the major glucocorticoid in rats, was administered at doses that restored its physiological levels. These results provide strong evidence that the ADX effect in PTX rats is the result of the loss of corticosterone. The Ca-lowering action of corticosterone appears to be a true glucocorticoid effect, since the potencies of synthetic and natural glucocorticoids, as indicated in bioassays in PTX-ADX rats, were in the same order as those established in assays of glycogen deposition in rats and of anti-inflammatory action in humans *(15)*.

The observation that serum Ca was unchanged after adrenalectomy or administration of even high doses of glucocorticoids in parathyroid intact (PTI) rats led us to the present study. We initially examined the interrelationship between glucocorticoids and parathyroid hormone (PTH). However, during the course of the study, the findings indicated strongly that calcitonin (CT) was also involved. First, an ADX effect in rats was observed in rats given hypercalcemic doses of PTH. Second, the ADX effect occurred only in the absence of the thyroid gland (-CT). Our results show that glucocorticoids, in addition to lowering Ca in PTX rats, also limit the hypercalcemia induced by PTH or calcitriol. Although endogenous glucocorticoids can act to restrict hypercalcemia, such action, under these experimental conditions, is probably secondary to that of CT.

Results

Examination of the ADX Effect

Previous experiments by Williams et al. *(13)* and by us *(14)* showed no change in serum Ca 4–6 h after ADX in rats with intact parathyroid glands. Similarly, serum calcium did not increase 5 h after thyroidectomy (TX), eliminating the source of CT, in rats with homologous functional parathyroid transplants (PTT) *(16)*, although a small transient increase after TX has been reported *(17)*. However, to our knowledge, no one had previously examined the effects on blood Ca after removal of both the adrenal and thyroid glands in rats, eliminating two serum-Ca-lowering agents, corticosterone and CT, respectively. We found that neither ADX, TX, nor both ADX and TX in rats with functional PTT affected the 5-h serum ionized or total Ca values (data not shown). Clearly, no ADX effect occurred whether the thyroid gland was present or not.

Examination of Glucocorticoid Effect

In another experiment, we examined the Ca-lowering effect of hydrocortisone (cortisol) in ADX rats either with intact thyroid and parathyroid glands or in thyroid intact (TI) and TX rats with functional PTT. The sc injection of cortisol, 0.2 mg/rat, given just after the operation(s) did not result in a significant 5-h fall in serum Ca in PTI and TI rats after ADX (Fig. 1). Similarly, cortisol did not lower serum Ca in ADX-PTT rats with or without the thyroid gland (Fig. 1). In contrast, the glucocorticoid effect was quite evident in rats after both ADX and thyroparathyroidectomy (TPTX)

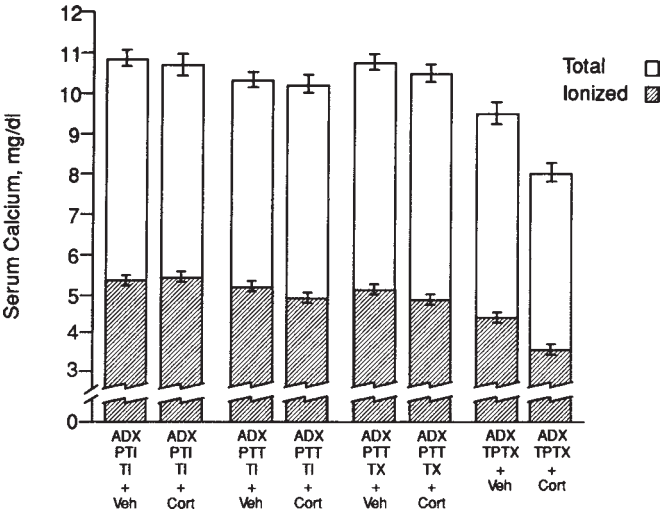

Fig. 1. The 5-h effect of cortisol (Cort) compared to vehicle (Veh) in ADX rats with intact parathyroid glands (PTI) or with functional PT transplants (PTT), and in TPTX rats. There were 3–6 rats/group. Cortisol, 0.2 mg/rat given sc immediately after the operations, failed to lower serum Ca significantly except in TPTX rats ($P < 0.01$). See text for details of the experimental protocol. In this and Figs. 3–8 the heighth of the bars represent the mean value and vertical brackets represent the SE.

(Fig. 1, the last two bars on the right); the glucocorticoid effect was somewhat expected, since we had previously demonstrated that an adequate dose of cortisol abolished the ADX effect in rats after PTX *(14)*.

Dose–Response: Rat Parathyroid Hormone (rPTH 1-34) and Serum Ca in TPTX Rats

The results obtained so far suggest that in PTI rats either the presence of a functional parathyroid gland or the normal level of serum Ca prevented the ADX effect as well as the glucocorticoid effect (Fig. 1). To distinguish between these two possible mechanisms, the endogenous source of PTH was removed by PTX and replaced by sc rPTH 1-34. Appropriate doses were established by determining the dose–response between rPTH and serum calcium in TPTX rats. The thyroid gland was also removed since the endogenous secretion of CT restricts the development of hypercalcemia in PTH-treated rats *(16)*.

Immediately after TPTX, rats, fasted overnight, were injected sc with rPTH 1–34 and injected again 2.5 h later. The experiment ended when the rats were bled 5 h after the operation. After establishing an appropriate dose range in preliminary experiments, three consecutive experiments were performed. The results from the first experiment are shown in Fig. 2. As can be seen, a dose of 0.08 μg/100 g body wt of rPTH 1-34, given twice, increased the 5-h serum Ca significantly ($P < 0.05$ and $P < 0.01$, total and ionized, respectively) above the low level of the TPTX rat not injected with PTH. Serum Ca rose further with higher doses. There was a highly significant ($P < 0.003$) linear dose–response relationship between doses of rPTH 1-34,

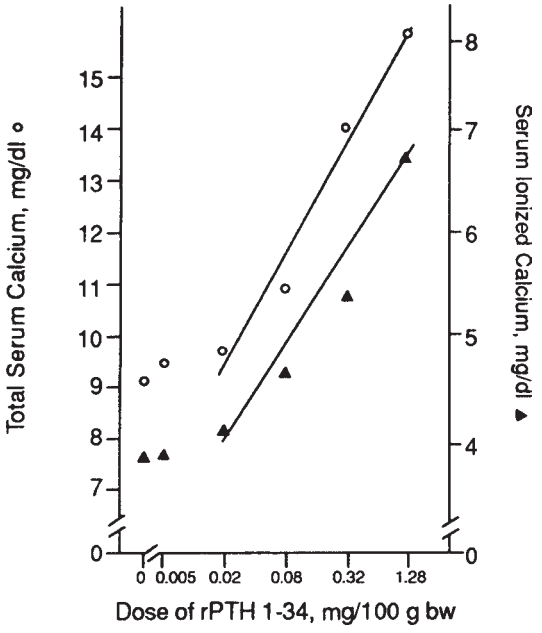

Fig. 2. Dose-response relationship between rPTH 1-34 vs total and ionized serum Ca. rPTH 1-34 was injected sc immediately after TPTX and again 2.5 h later. The rats were bled 5 h after PTH. There were 5 rats/group.

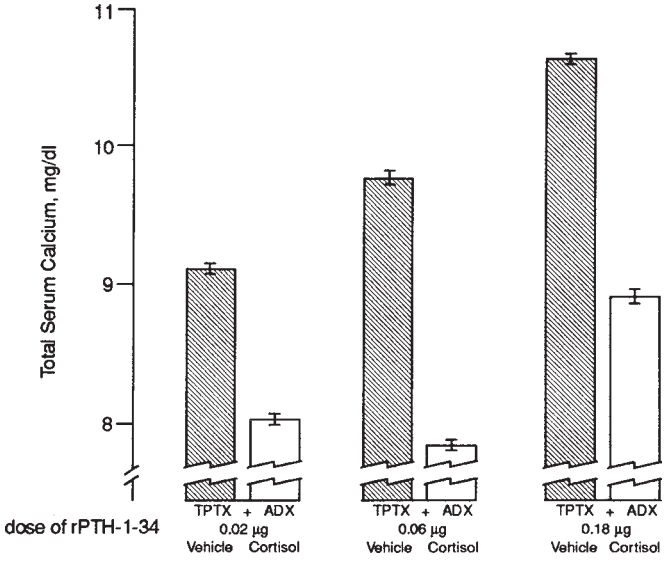

Fig. 3. The Ca-lowering effect of cortisol in TPTX-ADX rats treated with three different doses of rPTH 1-34. Male SASCO rats, fasted overnight, were subjected to TPTX and injected sc with 0.02, 0.06, and 0.18 μg immediately after the operation and again 2.5 h later. Rats in each dose group were injected sc with vehicle or 0.2 mg of cortisol immediately after the operations. Serum Ca was measured in rats, 5–6 rats/group, bled 5 h after the operations.

Table 1

Linear Function Between Dose of rPTH 1–34 and Serum Ca in Three Consecutive Experiments with TPTX Rats[a]

	Constant	Slope	λ
Total Ca			
Exp. 1	12.3	2.7	0.29
Exp. 2	12.9	3.7	0.27
Exp. 3	15.4	3.6	0.26
Mean values ± SE	13.6 ± 1.0	3.3 ± 0.3	
Ionized Ca			
Exp.1	6.2	1.6	0.26
Exp. 2	6.3	1.7	0.31
Exp.3	6.5	1.5	0.29
Mean values ± SE	6.4 ± 0.1	1.6 ± 0.6	

[a]For experimental details, *see* Fig. 1 and text.

0.02–1.28 μg/100 g body wt, and both ionized and total serum Ca. Linear relationships for the three consecutive experiment were determined. As shown in Table 1, there was good agreement among the constants, slopes, and indices of precision, λ (standard deviation divided by the slope). Using the mean values of the three constants and slopes, the following straight-line equations relating serum Ca to the dose of rPTH were calculated:

$$\text{Total serum Ca} = 13.6 + 3.3 \times (\text{log-dose rPTH 1-34}) \quad (1)$$

$$\text{Ionic serum Ca} = 6.4 + 1.6 \times (\text{log-dose rPTH 1-34}) \quad (2)$$

According to these equations, 0.08 and 0.13 μg/100 g body wt of rPTH, injected at 0 time and at 2.5 h are appropriate doses to maintain total Ca and ionized serum Ca at 10.0 and 5.0 mg/dL, respectively, 5 h after TPTX.

Glucocorticoid Response in rPTH-Treated TPTX-ADX Rats

In the next experiment, endogenous PTH was replaced with exogenous PTH in order to test the hypothesis that PTH in intact rats was responsible for preventing the Ca-lowering effect of cortisol. Immediately after ADX and TPTX, the rats were injected sc with vehicle or 0.2 mg of cortisol and with three dose levels of rPTH 1–34, 0.02, 0.06, or 0.18 μg/100 g body wt. The rPTH was injected again 2.5 h later. All rats were bled 5 h after the operations. The results shown in Fig. 3 revealed that glucocorticoid-treated rats had lower levels of serum Ca than vehicle-treated rats ($P < 0.001$), even in those rats given the highest dose of PTH, one that resulted in total serum Ca values well above 10 mg/dL (Fig. 3). The glucocorticoid effect was unexpected, because it was absent in rats with functional parathyroid glands (Fig. 1). The results show clearly that normal or high levels of serum Ca did not inhibit the glucocorticoid effect. Possible explanations for these findings were explored in the following experiments.

Glucocorticoid Effect in Rats Treated with Bovine PTH (bPTH)

That the entire 1-84 sequence of PTH might be required to inhibit the glucocorticoid effect was tested by the use of crude acid extracts of Bovine Parathyroid Substance. The rats were injected sc twice with 0.5 mL of the extract, representing 22.5 mg of Bovine Parathyroid Substance. Some rats were injected with cortisol, 0.2 mg/rat, whereas the

remainder received vehicle. Five hours after TPTX-ADX serum Ca in rats treated with vehicle increased to 12.4 ± 0.3 (SE) mg/dL, whereas serum Ca in those treated with cortisol increased only to 11.1 ± 0.3 mg/dL (data not shown). The inhibition by cortisol was highly significant ($P < 0.001$). These results did not differ from those in Fig. 3, and indicate that the ADX and glucocorticoid effects were the same whether rPTH 1-34 or bPTH 1-84 was used to replace endogenous PTH after TPTX. Several other experiments using extracts prepared from fresh bovine parathyroid glands also confirmed these findings.

Infusion of rPTH into TPTX Rats

To examine whether or not the short half-life of injected PTH is a factor in the observed glucocorticoid effect, rPTH 1-34 was infused with Alzet pumps into female rats just after TPTX. The dose, 0.055 µg/h, sufficient to maintain blood rPTH levels above normal, was based on units of rPTH 1-34 similar to that of bPTH 1-34 used by Ibrahim et al. *(18)* and Jaeger et al. *(19)* as calculated from relative potency estimates obtained from Keutmann et al. *(20)* using in vitro assays. It is of interest that the total dose of rPTH infused over the 5-h interval was not much below that given by the two sc injections over the 5-h interval (0.27 µg/5 h infused into a 150 g rat vs the hypercalcemic dose of 0.36 µg/5 h in two injections sc/100 g body wt used in Fig. 3). After the sc insertion of the pump, the rats were fasted until the next morning, about 18 h later, when initial blood samples were taken. Then the rats were adrenalectomized and injected with cortisol, 0.2 mg/rat, or with vehicle. A second blood sample was taken 5 h later. As shown in Fig. 4, serum-ionized Ca rose significantly ($P < 0.001$) in the vehicle-treated rats compared to the zero time control. More important to this study was the finding that cortisol completely inhibited the ADX effect. These results showed that neither the ADX effect nor the glucocorticoid effect was affected by maintaining a high level of rPTH. Thus, the short half-life of injected rPTH was not a factor in the results shown in Fig. 3. Obviously, the Ca-lowering effect of cortisol (Fig. 3) was, in fact, the result of preventing the ADX effect.

Role of the Thyroid and Parathyroid Gland in the ADX Effect

The role of the thyroid and parathyroid gland in the ADX effect was examined by comparing the ADX and glucocorticoid effects in TPTX and in PTX rats with intact thyroid glands. This kind of experiment was essential, since all of the results shown in Figs. 2–4 were performed in TPTX rats. Male rats were injected sc with rPTH 1-34, 0.8 µg/100 g body wt, just after PTX, TPTX, PTX + ADX, or TPTX + ADX. The PTH was injected again 2.5 h later, and the rats bled at 5 h. As shown in Fig. 5, the ADX effect was absent in rats with intact thyroid glands. However, in TPTX rats' serum Ca increased markedly ($p < 0.02$) and a significant ADX effect was observed ($P < 0.05$, total serum Ca; $P < 0.01$ ionized serum Ca). These results demonstrate once again the well-known inhibition of serum Ca-raising effect of PTH by CT in thyroid-intact rats *(16)*. More important is that this experiment shows that the ADX effect in PTH-induced hypercalcemic rats was seen only when the thyroid gland was also absent.

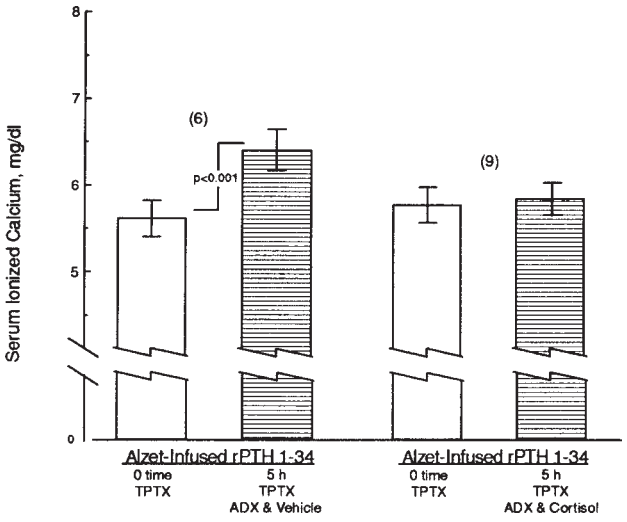

Fig. 4. Hypercalcemic response to ADX in TPTX rats infused with rPTH 1-34 and its inhibition by cortisol. For experimental details, *see text*.

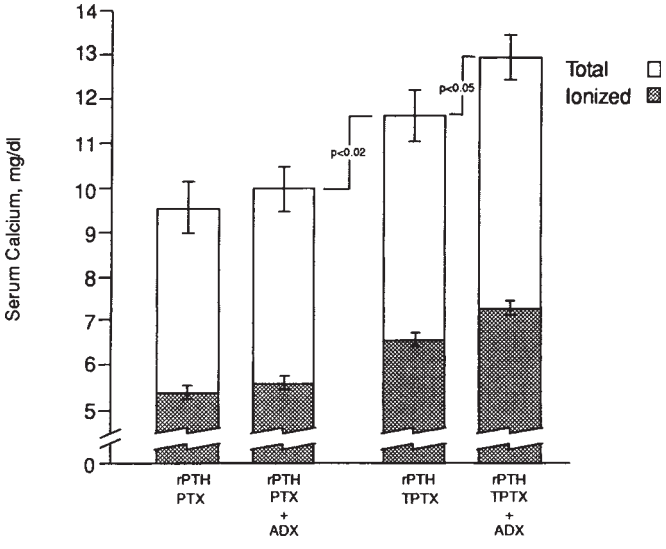

Fig. 5. Hypercalcemic response to ADX in PTX and TPTX rats treated with rPTH 1-34. Holtzman male rats, fasted overnight, were subjected to either PTX or TPTX, and $^1/_2$ of each group also ADX. The rats were injected with rPTH 1-34, 0.8 µg/100 g body wt, immediately after surgery and again 2.5 h later. All rats were bled 5 h after surgery. There were 5 rats in each group.

PTT and the ADX Effect

Since neither the ADX effect nor the glucocorticoid effect was observed in normocalcemic TI rats with PTT transplants (Fig. 1), the possibility exists that intact parathyroid glands secrete another agent, one that inhibits the ADX effect or the glucocorticoid effect. To test this possi-

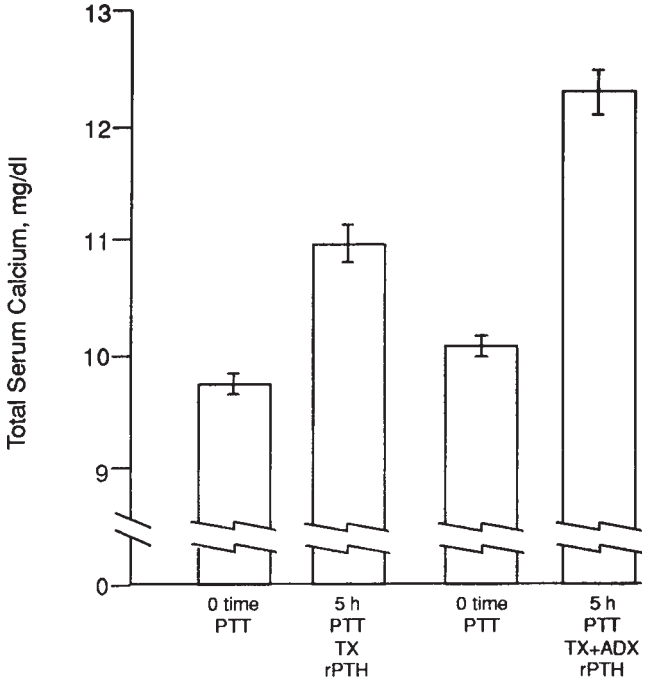

Fig. 6. The effect of functional PTT on the ADX effect in male rats treated with rPTH 1-34. Holtzman rats with PTT for 7–9 d were fasted overnight, bled (zero time), TX ($n = 11$) and TX+ADX ($n = 9$), injected sc with rPTH 1-34, 1 µg/100 g body wt, at zero time and 2.5 h later. Two separate experiments with comparable results were combined. The TX effect as well as the difference between TX and TX + ADX were significant at $P < 0.001$.

bility, we used rats with PTT. The result shown in Fig. 6 compared the effect of rPTH-induced hypercalcemia in PTT rats with and without thyroid glands. As in Fig. 5, TX resulted in a marked increase in serum Ca ($P < 0.001$). However, more important is the observation that even in the presence of functional PTT, the 5-h ADX effect still occurred in TX rats ($P < 0.001$ when bar 2 was compared to bar 4). The results show that under these conditions, the presence of functional PTT did not prevent the occurrence of the ADX effect. These findings do not support a role for the parathyroid gland in inhibiting either the ADX or the glucocorticoid effect.

Cortisol Effectively Blocks the ADX Effect

Evidence that glucocorticoids can reverse the ADX effect, but not the effect of the absence of CT is presented in Fig. 7. A marked hypercalcemic effect was observed in female rats treated with four sc injections of 0.3 µg/100 g body wt of rPTH 1-34 after TPTX and ADX, but not in TI rats after PTX and ADX. These results once again demonstrate the capacity of the intact thyroid gland to restrict hypercalcemia. Cortisol reduced only part of the hypercalcemia in the TPTX-ADX rats (last bar on the right), indicating that cortisol reversed only that part of the hypercalcemia owing to ADX. This experiment shows once again the lack of a glucocorticoid effect in TI rats (Fig. 7).

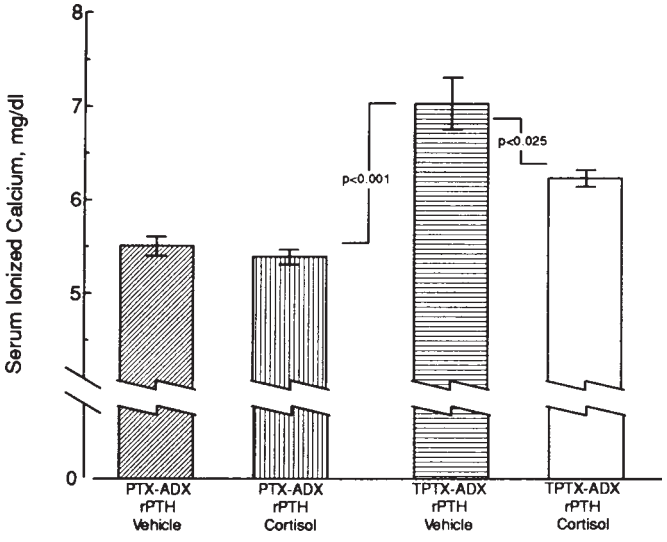

Fig. 7. The cortisol-inhibition part of the hypercalcemic response to ADX in TPTX rats given rPTH 1-34. Female Holtzman rats were subjected to ADX and either PTX or TPTX. All of the rats were injected with 0.3 µg/100 g body wt of rPTH immediately after the operations and again $1^1/_4$, $2^1/_2$, and $3^3/_4$ h later. The rats were given either vehicle or cortisol, 0.2 mg/rat, immediately after the operations. All rats were bled at 5 h. There were 5–6 rats/group.

Adrenals Effectively Block Calcitriol-Induced Hypercalcemia

Finally, we wondered if the ADX effect in TX rats was limited to PTH-induced hypercalcemia or if it could also be observed during calcitriol treatment. The next experiment, depicted in Fig. 8, showed that in calcitriol-treated female TPTX rats, serum Ca increased significantly ($P < 0.001$) 5 h after ADX (last bar on the right). In contrast, in rats with intact parathyroid and thyroid glands, serum Ca was not altered by either ADX or cortisol.

Discussion

Initially, in examining the interaction between glucocorticoids and PTH, we did not believe that CT was implicated, because in our earlier study, the presence or absence of CT did not alter the ADX effect. For example, the ADX effect occurred in rats whether they were parathyroidectomized by surgical excision (thyroid intact), by TPTX (removing the source of CT as well as PTH), or by cautery (excessive release of CT as well as removing the source of PTH) *(14)*. Thus, during the development of hypocalcemia after PTX, CT is not a factor in either the ADX effect or in the glucocorticoid effect shown to prevent the ADX effect in PTX-ADX rats.

The present study began by examining why neither the ADX effect nor the glucocorticoid effect occurred in rats with intact parathyroid glands. We wondered if removal of both the thyroid and adrenal glands, the source of two Ca-lowering agents, CT and corticosterone, might lead to an increase in blood Ca in rats with functional PTT. However,

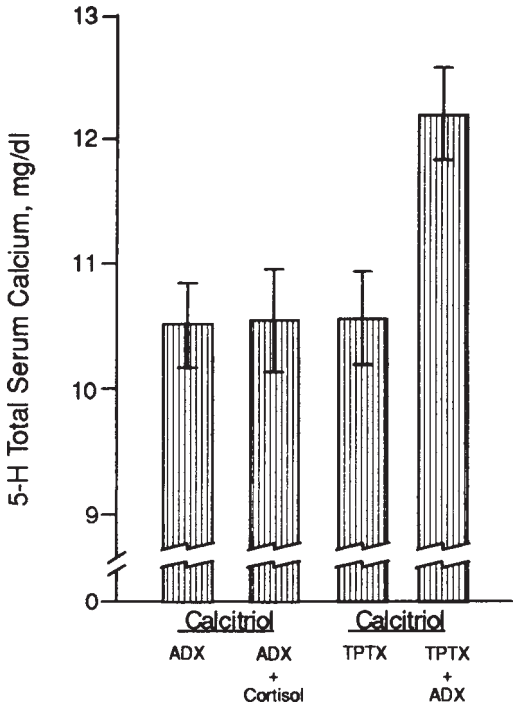

Fig. 8. Hypercalcemic response to ADX in TPTX rats treated with calcitriol. Female rats were injected ip with 6 μg of calitriol 19 h before the beginning of the experiment. After an overnight fast, the rats were bled and subjected to TPTX, ADX, or both TPTX and ADX. The rats were bled 5 h later. Adrenalectomy led to a highly significant increase in serum Ca ($P < 0.001$) only in TPTX rats. There were 4–5 rats/group.

removal of both glands did not result in a rise in serum Ca. Thus, in normocalcemic rats, neither the thyroid nor adrenal glands appear to be acting to keep blood Ca on the low side.

The role of PTH in the inhibition of both the ADX and glucocorticoid effects was examined by giving exogenous PTH to rats after TPTX. The removal of the thyroid gland, to eliminate the antihypercalcemic action of endogenous CT in PTH-treated rats *(16)*, led to the unexpected finding; cortisol effectively lowered serum Ca in TPTX rats treated with doses of rPTH 1-34 that were hypercalcemic. Thus, neither normal nor high blood Ca was responsible for the lack of the cortisol response in PTT rats. Other experiments with sc administered bPTH 1-84 gave comparable results to rPTH 1-34, thereby ruling out the possibility that the lack of the C-terminal fragment of the 84 amino acid hormone was a factor in the response. Also, when rPTH 1-34 was infused continuously with osmotic pumps, the results obtained were no different than those when PTH was injected, ruling out the short half-life of injected rPTH as a factor.

Having ruled out PTH and blood Ca as factors in the absence of the glucocorticoid effect, we turned to examining the role of the thyroid gland. The experiments (Figs. 5–7) showed clearly that there was an ADX effect in PTH-treated rats only if the rats were thyroidectomized and that the ADX effect could be prevented by treatment with cortisol (Figs. 4 and 7). Thus, the observed glucocorticoid effect as seen in Fig. 3 was, in reality, the prevention of the ADX effect. From these findings, we concluded that endogenous CT masks the antihypercalcemic effect of endogenous glucocorticoids.

Our findings may be relevant to the occasional appearance of hypercalcemia of adrenal insufficiency *(3,4)*. In a study of 81 cases of adrenal insufficiency, 20% of the subjects had episodes of hypercalcemia *(21)*, an indication that its occurrence is not infrequent. The mechanisms responsible for the appearance of the hypercalcemia remain unclear. We suggest that those adrenal-insufficient patients who exhibit hypercalcemia may either have a deficiency of calcitonin or a defect in CT responsiveness.

The mechanisms of the glucocorticoid effects on blood Ca remain to be elucidated. However, the rapid 5-h response suggests that glucocorticoids, like CT, act on bone. Glucocorticoid receptors have been identified in bone *(22)*. Also. it is unlikely that the rapid 5-h serum Ca responses to ADX or glucocorticoids in fasted rats is owinge to renal or gastrointestinal actions. Although ADX has been shown to reduce the urinary excretion of Ca in PTX rats *(11)*, the urinary Ca excretion in our fasted rats is very low and not significantly altered by either PTX or ADX. Also, in vitro experiments by Stern *(23)* indicated a direct action of glucocorticoids on bone. Preliminary evidence *(24)* using ^{45}Ca in experiments similar to those by O'Riordan and Aurbach *(25)* indicated that glucocorticoids inhibit the movement of Ca from bone to ECF. Much earlier studies by Talmage et al. *(26,27)* also suggested that glucocorticoids, like CT, inhibited Ca release from bone.

The results of this study have not revealed the reason why neither ADX nor exogenous glucocorticoids altered serum Ca levels in normocalcemic parathyroid-intact rats or in rats with functional PTT. However, the finding revealed a calcitonin-like antihypercalcemic action of endogenous glucocorticoids not previously known. Thus, this study emphasizes further the contrasting roles of CT and glucocorticoids in normocalcemic, hypocalcemic, and hypercalcemic conditions. In intact normocalcemic rats, exogenous CT *(28)*, but not glucocorticoids *(10,13,14)*, is hypocalcemic. After PTX, both CT and glucocorticoids are hypocalcemic.

Our study did not reveal why neither ADX nor glucocorticoids affected blood Ca in normocalcemic rats with intact parathyroid glands. That an increase in rPTH secretion might compensate for a Ca-lowering action of cortisol was ruled out; serum levels of rPTH determined using immunoradiometric homologous two-site assays *(29)* were unaltered by high sc doses of cortisol between 15 min and 5 h in adrenal-intact and ADX rats (unpublished experiments). In contrast to normocalcemic PTI rats, there were marked Ca-lowering effects of exogenous glucocorticoids in hypercalcemic ADX-TPTX rats. Apparently, glucocorticoids modulate blood Ca only under conditions where Ca metabolism is perturbed. Further studies must be undertaken to elucidate the importance of glucocorticoids as well as CT in the normal physiology of Ca metabolism.

We conclude that during induced hypercalcemia, both CT and glucocorticoids are antihypercalcemic, but the antihypercalcemic function of glucocorticoids appears be secondary to that of CT.

Materials and Methods

Chemicals and Reagents

Cortisol was purchased from Sigma Chemical Co. (St. Louis, MO). rPTH 1-34 was purchased from Bachem California (Torrance, CA). Bovine Parathyroid Substance, a powder prepared by Wilson Laboratories, Lot #146729, was obtained through the kindness of Armen H. Tashjian and Edward F. Voelkel. Cortisol was dissolved in 95% ethanol, 1 mg/0.1 mL ethanol, and diluted further with acid-saline, an acidified physiological salt solution (0.001 *N* HCl-0.9% NaCl). The stock solution of rPTH 1-34, 200 μg/mL, was prepared by dissolving 1 mg of hormone in 0.2 mL of 0.1*N* HCl, diluted with 4.8 mL of 0.001*N* HCl, and stored frozen. Further dilutions were made with acid-saline, and the final solution for sc injections and for continuous infusions with Alzet pumps contained 2% heat-inactivated rat serum *(30,31)* and 2% cysteine *(18,32)*, respectively. The calcitriol was provided to Svein Toverud by Milan Uskokovic of Hoffman-LaRoche (Nutely, NJ).

Animals and Diets

Most of the experiments were performed on male and female Holtzman rats, about 1 mo old, born in our animal facility from pregnant females. Other rats were purchased from Harlan Sprague Dawley Inc. (Holtzman), Indianapolis, IN and from SASCO, Madison, WI. They were fed Purina Rodent Laboratory Chow #5001 until the beginning of the experiment. There were no apparent differences in results between male and female rats *(14)* or between rats obtained from different sources.

Surgical Procedures and Protocols

All surgical operations were performed on rats anesthetized with ether. PTX by surgical excision and TPTX have been described previously *(33)*. Homologous PTT were performed in some rats as described by Tashjian *(34),* except that immediately after removal from their capsules the glands were placed, without rinsing in Gey's solution, into pockets made by a longitudinal slit in the scalinus medius muscle. TX was performed in some of the rats in which the parathyroid glands had already been removed and transplanted, whereas in other rats, the thyroid gland was left intact. Only those rats with functional PTT as determined 7–14 d later, with zero time serum Ca value at or above 9 mg/dL after an overnight fast, were retained in the experiment. Bilateral ADX was performed through a single dorsal incision *(35)*.

The rats in most of the experiments were fasted overnight, and, about 9 AM, the rats were anesthetized with ether, bled by retro-orbital sinus puncture, subjected to the operation(s), and injected sc with rPTH and with either vehicle or cortisol, 0.2 mg/rat. Five hours later, the rats were anesthetized again and bled. Other modifications in protocols are described in the legends to the figures.

In two experiment (Fig. 4), rPTH was infused with osmotic minipumps. Alzet pumps, model 2001, Alza Corp., Palo Alto, CA, to deliver 1 μL/h for up to 7 d, were filled with solutions of rPTH 1-34. The pumps were incubated overnight at room temperature in isotonic saline and then inserted in fed rats immediately after TPTX through a small ventral incision and forced sc caudally to the nape of the neck. The incision was closed with wound clips. The experiment began the next day at about 9 AM when the rats, fasted overnight, were anesthetized with ether, bled by retro-orbital sinus puncture, adrenalectomized, and then injected sc with either vehicle or cortisol, 0.2 mg/rat. Five hours after the operation, the rats were anesthetized, bled again, and the pump removed. The pump was then inserted into a new recipient rat just after TPTX. Since the rats were assigned to groups by randomizing from the heaviest to the lightest rats and using the heaviest rats first, the weights of rats at the time used during the 3 d of the experiment were similar.

Total serum Ca was determined by a fluorometric method *(36)*. Ionized serum calcium was measured by the method of Thode et al *(37)* with a Radiometer ICA 1(Copenhagen, Denmark).

The animal procedures employed in this study were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

Statistical Analyses

The data in each experiment were subjected to analysis of variance. The standard errors shown in Figs. 1–6 and Table 1 were calculated from the residual error term of the appropriate analysis of variance. In the experiment reported in Fig. 7, the variances were heterogeneous. The significance of differences between mean values with three or more groups was estimated by the method of Scheffé *(38)*. In some experiments, Figs. 4, 6, and 8, paired differences, the change in serum Ca between the zero time and 5 h, were used to determine significant effects of treatment. When means of only two groups were compared, the Student's *t*-test was employed. A probability value of 0.05 was taken arbitrarily to represent a significant difference.

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