Endocrine, *vol. 6, no. 3, 279–283, June 1997* 0969-711X/97/6:279–283/$9.25

Calcium-Lowering Action of Glucocorticoids in Adrenalectomized-Parathyroidectomized Rats

Specificity and Relative Potency of Natural and Synthetic Glucocorticoids

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The specificity and potency of glucocorticoids to lower serum calcium (Ca) in rats after parathyroidectomy (PTX) and adrenalectomy (ADX) were examined. Rats fasted overnight were given sc injections of various steroids immediately after the operations. The fall in serum calcium 5 h after PTX-ADX in rats given hypocalcemic doses of corticosterone was compared to that after injection of a test steroid. At high doses, progesterone, estradiol, testosterone, and aldosterone were inactive, whereas glucocorticoids were consistently hypocalcemic. These results indicate that the Ca-lowering effect is specific for steroids with glucocorticoid activity. Potency estimates were made by comparing the dose-response of natural and synthetic glucocorticoids to that of corticosterone, the major glucocorticoid in rats. The mean potency of hydrocortisone was 8.2 times that of corticosterone. Prednisolone was about 9.6, triamcinolone 33, betamethasone 109, and dexamethasone 301 times as potent as corticosterone. Thus, the use of the calcium-lowering action as a bioassay has provides a specific and rapid in vivo method to compare potencies of glucocorticoids consistent with those obtained by anti-inflammatory and glycogen deposition assays. The importance of this interesting calcitonin-like action of glucocorticoids in normal physiology of calcium metabolism is not yet established.

Key Words: Glucocorticoids; bioassay; potency; hypocalcemia; calcitonin-like action.

Introduction

The fall in serum calcium (Ca) after parathyroidectomy (PTX) in rats has been shown to be markedly reduced by adrenalectomy (ADX) *(1–6)*. In our previous paper, we demonstrated that the ADX effect, the higher serum Ca in young (100 g) male or female PTX-ADX rats than after PTX alone, was abolished by restoring normal levels of corticosterone, the major glucocorticoid in rats *(6)*. On the basis of these findings, we concluded that the loss of endogenous glucocorticoids was responsible for the ADX effect after PTX. Clearly, endogenous corticosterone is responsible for part of the rapid fall in serum calcium after PTX *(6)*.

In this study, we examined the specificity of the hypocalcemic response in ADX-PTX rats to steroids with and without glucocorticoid activity. Only glucocorticoids were found to be hypocalcemic. The potency of natural and synthetic glucocorticoids to lower serum Ca was compared to that of corticosterone. We found that the order of potency of glucocorticoids estimated by this new and simple in vivo Ca-lowering bioassay is the same as that established earlier in assays by anti-inflammatory and glycogen deposition assays.

Results

The 5-h hypocalcemic response of glucocorticoids to lower serum Ca of the ADX-PTX rat is the basis of the bioassay. The ADX effect in PTX rats was usually between 0.7 and 1.5 mg total serum Ca/dL. The larger the ADX effect, the easier it was to determine dose–response relationships. Figure 1 shows the result of one bioassay comparing responses of triamcinolone and dexamethasone to corticosterone. The ADX effect, the 5-h difference between PTX alone and ADX-PTX shown on the right of Fig. 1, was 1.26 mg/dL ($P < 0.001$). The potency estimated by parallel-line bioassay statistics for triamcinolone and for dexamethasone compared to corticosterone was 33 and 300, respectively.

In another bioassay in which the potency of hydrocortisone was compared to that of corticosterone, both total and ionized serum calcium values were determined (Table 1). Total serum Ca in the ADX-PTX rats was higher, 8.3 mg/dL, compared to that after PTX alone,

Received December 26, 1996; Revised March 3, 1997; Accepted March 12, 1997.

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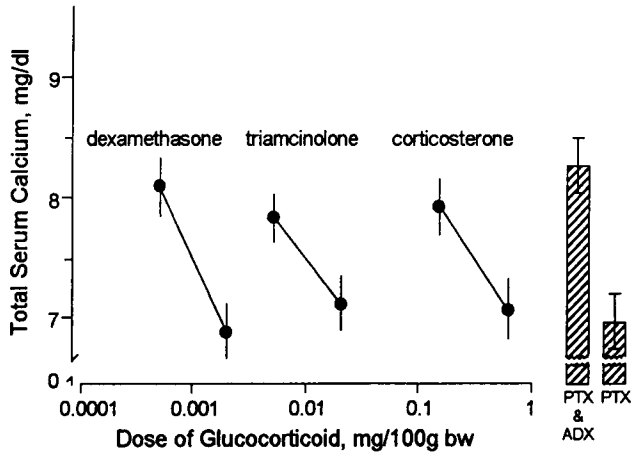

Fig. 1. Bioassay of dexamethasone and triamcinolone compared to corticosterone as standard. Each steroid was administered sc at two dose levels just after ADX and PTX, and the rats were bled 5 h later. The points and vertical lines represent the mean and SE, respectively, of serum Ca values for 4–6 rats. The height of each bar and vertical line on the right represents the mean and SE, respectively, of serum Ca in uninjected controls, 5 rats/group.

6.9 mg/dL, a difference of 1.4 mg/dL, significant at $P < 0.001$. Although the highest dose of corticosterone did not lower serum Ca to the same level as PTX alone, the highest dose of hydrocortisone drove the serum Ca slightly lower than PTX alone. Similar results were obtained using serum-ionized Ca, except that the values were about 50% those of total serum Ca. Bioassay statistics performed on the results summarized in Table 1 revealed a potency for cortisol of 9.8 (total serum Ca) and 9.6 (ionized serum Ca) times that for corticosterone (Table 2).

Some steroid hormones that are not glucocorticoid were tested for hypocalcemic activity in the ADX-PTX rat. As shown in Table 3, aldosterone, β-estradiol, progesterone, and testosterone were not hypocalcemic at the doses tested.

Table 4 summarizes potency estimates and 67% confidence limits for a number of glucocorticoids. Estimates obtained from both ionic and total Ca values in the same assay were very close. The mean potency of hydrocortisone measured in two separate assays was 8.2 times as potent as corticosterone. Prednisolone was 9.6, triamcinolone 33, betamethasone 109, and dexamethasone 301 times as potent as corticosterone.

Discussion

The results of this study demonstrate that the Ca-lowering action in rats after PTX and ADX is highly specific for steroids with glucocorticoid activity. Further, the order of potency for steroids (Table 4) as determined by their Ca-lowering activity was essentially identical to that obtained in classic assays, such as glycogen deposition in rats and anti-inflammatory activity in humans (Tables 63.2 and 63.3, Haynes and Murad *[7]*). These results strongly suggest that the hypocalcemic response represents an authentic glucocorticoid-type action. Also demonstrated is the utility of this bioassay to provide a simple and rapid method to compare potencies of glucocorticoids in vivo.

In contrast to the agreement in the order of relative potency, there were marked differences in potency ratios between our assays and the classic assays. For example, the potency for cortisol was eight times as potent as corticosterone, whereas it was about three times as potent in the liver glycogen and anti-inflammatory assays *(7)*. Previously, using slow-release pellets as the method of administration of steroids, we had estimated that cortisol was 35 times as potent as corticosterone *(6)*. Potency estimates apparently depend, in part, on the method of testing, but the marked difference in this assay method between these steroids may reflect an unusual sensitivity to hydrocortisone, owing perhaps to its absence in rats.

The recognition that very high doses of glucocorticoids used in therapy as immunosuppressive and antiinflammatory agents induce bone loss and osteoporosis *(8)* has led to the assumption that glucocorticoids, in general, are harmful to bone. However, the hypocalcemic effect of glucocorticoids in our studies represent the opposite kind of response one would expect from an agent that causes bone loss. That endogenous glucocorticoids may inhibit bone loss has not received adequate consideration. In this regard, two recent publications relate to this discussion. Mora et al. *(9)*, studying young patients with congenital adrenal hyperplasia given long-term (15 ± 4 yr) replacement glucocorticoid therapy, found that bone mineral density measurements of spine, total body, legs, and arms were not significantly different from those in age-matched controls. More pertinent are the findings of Li et al. *(10)*, who showed beneficial effects of replacement therapy in ADX rats. They found that 3-wk supplementation of corticosterone to near physiological levels in ADX rats prevented the development of cancellous osteopenia seen in placebo-treated ADX controls.

The physiological importance of the rapid effects of glucocorticoids on blood Ca shown in our study may be as difficult to establish as it has been for calcitonin *(11,12)*. One of the major problems in delineating a physiological role for glucocorticoids or calcitonin involves the failure to alter blood Ca by removal of either the adrenal gland in parathyroid-intact rats *(2,5,6,13)* or the thyroid gland in rats with functional parathyroid transplants *(14,15)*. Another enigma is that unlike calcitonin *(16,17)*, injecting glucocorticoids, even at high doses, into parathyroid-intact rats does not lower serum Ca *(2,5,6)*. Nonetheless, Talmage et al. *(18)* showed that administration of hydrocortisone to parathyroid-intact rats did indeed affect bone; although plasma Ca remained stable, hydrocortisone reduced the removal of radioactivity from bone in rats injected with ^{85}Sr two wk earlier. Talmage and Kennedy *(13)* also demonstrated that in rats given ^{45}Ca two wk earlier,

Table 1
Dose-Responses of Corticosterone and Hydrocortisone in Abolishing the ADX Effect in Female PTX Rats

Group	N	Treatment	Total Serum Ca mg/dL ± SE[a]	Ionized Serum Ca mg/dL ± SE[a]
1	6	PTX by surgical excision	6.9 ± 0.2	3.3 ± 0.1
2	5	PTX by surgical excision ADX	8.3 ± 0.2	4.0 ± 0.1
3	5	PTX by surgical excision ADX + 0.15 mg corticosterone/100 g body wt	8.3 ± 0.2	4.0 ± 0.1
4	5	PTX by surgical excision ADX + 0.60 mg corticosterone/100 g body wt	7.6 ± 0.2	3.6 ± 0.1
5	5	PTX by surgical excision ADX + 0.019 mg hydrocortisone/100 g body wt	8.3 ± 0.2	3.9 ± 0.1
6	4	PTX by surgical excision ADX + 0.075 mg hydrocortisone/100 g body wt	7.3 ± 0.3	3.5 ± 0.1
7	4	PTX by surgical excision ADX + 0.30 mg hydrocortisone/100 g body wt	6.3 ± 0.05	3.0 ± 0.01

[a]Standard errors were calculated from the residual term of the ANOVA from results in groups 1–6. The ADX effect was significant at $P < 0.001$. The SE of group 7 was calculated separately since the serum Ca values were compressed below the level of the PTX control, group 1, creating a significantly smaller variance. The mean weight ± SE of the rats was 114 ± 2.

Table 2
Potency of Hydrocortisone Compared to Corticosterone[a]

Serum calcium	Potency estimate	Confidence limits 67%	Standard deviation SD, mg/dL	Index of precision SD/b slope
Total Ca	9.8	6.2–17	0.34	0.22
Ionized Ca	9.6	5.1–22	0.23	0.32

[a]Parallel line bioassay statistics were performed on the results in groups 3–7 of Table 1. λ = SD/b,where b is the combined slopes of the dose-response curves.

ADX increased the removal of radio-activity from bone, again not affecting the plasma calcium in rats with intact parathyroid glands. From earlier studies it is apparent that in PTX rats, the two hormones have similar actions; glucocorticoids, like CT *(19)*, are hypocalcemic *(2,5,6)*, owing primarily to an action on bone *(20)*. We believe that our demonstration of specificity and ordered potency of the Ca-lowering action of steroids with glucocorticoid activity demonstrates a calcitonin-like role for glucocorticoids, results suggesting that glucocorticoids may have a beneficial role in Ca and bone metabolism.

Materials and Methods

Chemicals and Reagents

Corticosterone, hydrocortisone (cortisol), prednisolone, triamcinolone, β-methasone, dexamethasone, aldosterone, β-estradiol, progesterone, and testosterone were obtained from Sigma Chemical Co. (St. Louis, MO).

Animals and Diets

Male and female Holtzman rats, 4–6 wk of age, were fed Purina Rodent Laboratory Chow #5001 until 4:30 PM on the night before the experiment when food was removed from the rat cages.

Experimental Protocols

On the day of the experiment, 9 AM, the rats were anesthetized with ether, and one operator performed the PTX by excision *(21)* and another person, the ADX *(22)*. The two operations take < 5 min and operations and injections on 30–40 rats are completed within 3 h. The bioassay is based on the comparison of calcium-lowering action of test steroids to that of standard doses of corticosterone in the PTX-ADX rats. Each steroid was dissolved in a minimum amount of alcohol, usually 0.2 mL of 95% ethanol, and diluted further with water. The rats were injected sc with 0.5 mL of corticosterone standards, 0.15 mg and 0.6 mg/100 g body wt, test solutions, and diluent (controls) immediately after PTX and ADX. In each experiment, one group of uninjected PTX and ADX-PTX rats served as controls to verify the presence of a significant ADX effect. Preliminary experiments were performed to determine hypocalcemic doses of glucocorticoids that fell on the dose–response curve. All rats were bled by retroorbital sinus puncture 5 h after the operation(s). In some experiments, only total serum Ca was determined *(23)*, and in other experiments, only ionized calcium *(24)*. In some experiments, both ionized and total calcium were measured.

Statistical Analyses

The data were subjected to analysis of variance to identify significant responses *(25)* owing to the ADX effect

Table 3
Steroid Hormones Not Hypocalcemic in Male ADX-PTX Rats

Steroid hormone	Experiment	Dose[a], mg	Change in total serum Ca ± SE from ADX-PTX Controls, mg/dL
Aldosterone	1	0.1 mg	+0.20 ± 0.50
β-estradiol	2	1 mg	−0.45 ± 0.37
Progesterone	2	1 mg	−0.03 ± 0.35
Testosterone	3	1 mg	−0.01 ± 0.37

[a]Each steroid was administered sc at the dose indicated/100 g body wt immediately after PTX and ADX and blood drawn 5 h later. The lack of response of the above steroids was compared in the same experiment to the hypocalcemia induced by hydrocortisone (0.2 mg/100 g body wt). Aldosterone was administered at a lower dose because its endogenous levels are normally several orders of magnitude below those of the other steroid hormones tested.

Table 4
Potency of Natural and Synthetic Glucocorticoids Relative to Corticosterone

	Experiment	Potency estimate	Confidence limits 67%	Glycogen deposition in rats[a]	Anti-inflammatory in humans[a]
Corticosterone				1	1
Hydrocortisone	D Total Ca	9.8	7.9–12.5		
	D Ion Ca	9.6	7.0–13.7		
Mean ionic and total calcium assays			9.7		
Hydrocortisone	A Total Ca	6.8	4.5–12.3		
Mean of assays	A and D		8.8	2.9	3.3
Prednisolone	E Total Ca	9.6	7.2–13	11.4	13.3
Triamcinolone	C Total Ca	31	24–42		
	C Ion Ca	35	28–44		
Mean ionic and total calcium assays		33		14.3	16.7
Betamethasone	B Total Ca	109	75–166		71
Dexamethasone	C Total Ca	304	225–413		
	C Ion Ca	298	235–379		
Mean ionic and total calcium assay		301			71

[a]Modified from Tables 63-2 and 63-3, *The Pharmacological Basis of Therapeutics (7).*
In each assay, two doses of corticosterone were compared to two doses of triamcinolone and dexamethasone (Fig. 1, expt. C, females), β-betamethasone (expt. B, males), cortisol (expt. A, females), and to three doses of cortisol (Table 1, expt. D, females) and prednisolone (expt. E, males).

and to the injection of steroids. Potency of gluco-corticoids was estimated by statistical analysis for parallel line bioassay *(26)*. The bioassay software was provided by Robert C. Elston (Department of Biometry and Genetics, Case-Western Reserve, Cleveland, OH).

Acknowledgments

We thank Svein U. Toverud for advice during the course of this study and Robert C. Elston, Department of Biometry and Genetics, Case-Western Reserve, Cleveland, OH for the bioassay software as well as help and advice with the statistical analyses. We are also grateful to Terry Volz for the preparation of the figure and Renee B. Williams for help in the preparation of the manuscript.

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