Stimulation of Adrenal Corticosteroid Secretion by Hypercalcemia in the Dog (36221)

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The calcium ionic concentration of the adrenal milieu may significantly alter glucocorticoid secretion (1–3). Furthermore, calcium ions seem to be necessary for a number of hormonally mediated cellular events (4) including ACTH stimulation of adrenal glucocorticoid secretion (5).

Wide variations in adrenal arterial calcium concentration were made deliberately in an isolated perfused adrenal preparation. First, the acute effects of hypocalcemia induced by a divalent cation chelating agent were determined. Then, calcium was administered to restore the estimated calcium deficit and also to induce hypercalcemia. The secretion rates of steroid hormones, particularly glucocorticoids, were serially determined.

Materials and Methods. An isolated adrenal pouch was prepared according to the method of Hilton *et al.* (6). Two hypophysectomized dogs were used of which one provided the isolated adrenal pouch. The other served as the donor of arterial blood by terminal bleeding after bilateral nephrectomies, and adrenalectomies were carried out. Since the donor animal was infused with isotonic saline, the recipient pouch was perfused with somewhat diluted donor blood. After four 5-min collection periods to establish the base line secretion rates, Na_4 ethylenediaminetetracetic acid (EDTA) was added to arterial blood and was infused at 20 mg/min for 15 min (15 mg/min in Expts. 1 and 5). Then after 3 subsequent collection periods for recovery, calcium as the gluconate salt was added to the arterial blood and administered at the rate of 9 mg/min for 10 min (Fig. 1). Three collection periods after completion of the calcium administration, 5 units of bovine ACTH were injected rapidly (1 unit of ACTH in Expt. 1).

The arterial and venous plasmas were analyzed for calcium content by EDTA titration (7). The 17-OH corticosteroid (CS) concentrations were measured by the method of Peterson *et al.* (8). In Table I, are the venous hematocrits, measured in each collection period, expressed as mean ± SD, which did not change significantly during the course of each experiment. The means of the differences were compared and analyzed by Student's *t* test.

Results. Results of the calcium determinations are given in Table I. Since the hematocrits were low (mean, 25.6; range, 21–36%), it was evident that some dilution of the arterial blood had occurred in the collection process. The serum calcium fell during EDTA; in Table I are listed the calcium levels in the final 5-min collection period. A rapid rise in calcium through and beyond the physiological concentrations followed calcium infusion. After the Ca infusion was discontinued, Ca concentration fell swiftly, returning to base line during the ACTH administration.

The effects of EDTA, calcium and ACTH administration on the 17-OH CS secretion rates are presented in Table II. In each experiment the 3 base line collection periods were averaged. After the first 5 min of the EDTA injection, the 17-OH CS secretion rates in the next 3 collection periods were similarly averaged and showed no significant or consistent changes from the base line. However, beginning 5 min after calcium was started (period 2, Table II) the 17-OH CS secretion rates rose in all experiments, reaching a zenith in the period immediately after discontinuation of the calcium (period 3) and then falling back toward the base line. Subsequent ACTH administration produced its expected rise in 17-

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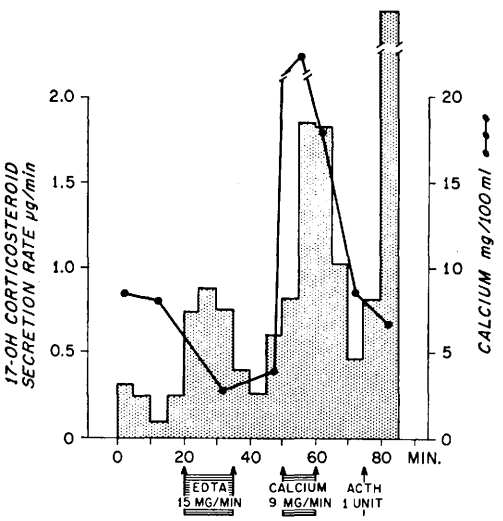

FIG. 1. Corticosteroid secretion rates plotted against time in a representative experiment: Timing of EDTA, calcium, and ACTH injections are indicated below the abscissa.

OH CS secretion.

The calcium effect on 17-OH CS secretion was present in the nonhypophysectomized animal (Expts. 8 and 9). Their high base line 17-OH CS secretion rates fell during EDTA, probably as a result of decreased residual ACTH activity in the recipient adrenal, although a specific EDTA inhibitory action cannot be excluded. Calcium administration, after the EDTA, then produced a rise in adrenal 17-OH CS secretion in both experiments, modest but significant in No. 8 and striking in No. 9.

In one experiment (No. 10 in Tables I and II), designed to test the hypothesis that the calcium effects on 17-OH CS secretion are also operative in the absence of prior EDTA administration, the order of EDTA and calcium administration was reversed. Calcium produced transient, but striking, increases above the high base line 17-OH CS secretion rates.

Discussion. Calcium in pharmacological

TABLE I. Effect of EDTA and Calcium on Calcium Concentrations in the Adrenal Vein Blood of the Isolated Adrenal Pouch Preparation (mg/100 ml) in Hypophysectomized Dogs (Group I) and Dogs with Residual Pituitary Functions (Group II).

Expt.	Control	EDTA	Recovery	Calcium			ACTH
				2[a]	3	4	
Group I							
1–7	7.1	4.0	6.2	55	17.6	12.0	7.0
N = 7	±1.3	±0.7	±2.1			±2.6	±1.2
Group II							
8	9.1	3.7	6.6	—	—	13	8.1
9	6.2	3.8	5.8	72	17	11.1	6.8
Expt.							
1–9							
N = 9							
Mean	7.2	3.9	6.4	61	17.5	12.1	8.7
SD	±1.4	±0.8	±1.9			±2.7	±1.9
Range	5.2–9.1	3.0–5.6	4.0–9.0			8.8–17.0	6.3–9.1

Group III[b]		Calcium			EDTA			
		1[a]	2	3	1[a]	2	3	
10	7.6 ±0.7	—	77	50	9	8.5	4.6	9.2

[a] Calcium concentrations in the first, second, third, or fourth 5-min collection period after starting the 10-min administration of calcium or EDTA.

[b] Reversed order of administration; calcium preceded the EDTA.

TABLE II. Effect of Calcium and EDTA on 17 OH Corticosteroid Secretion Rates by the Isolated Perfused Dog Adrenal Glands *in Situ*.

	Secretion rates (μg/min)						
	Control[a]	EDTA[a]	Recovery	Calcium[b]			
Expt.				2	3	4	ACTH
Hypophysectomized dogs							
1	0.23 ±0.13	0.56 ±0.27	0.60	1.79	1.73	1.03	3.75
2	0.50 ±0.18	0.60 ±0.21	0.47	0.90	0.62	0.43	3.40
3	0.46 ±0.33	1.90 ±0.70	1.51	1.66	2.37	2.52	3.41
4	1.23 ±0.15	1.13 ±0.15	1.11	1.64	1.85	0.74	1.74
5	0.31 ±0.15	1.38 ±0.56	0.89	0.81	2.19	1.99	1.93
6	0.59 ±0.22	0.51 ±0.27	0.44	0.61	0.75	0.40	2.67
7	1.75 ±0.22	1.01 ±0.57	1.62	2.36	2.65	2.32	2.92
1–7 mean	0.65	0.98	0.94	1.40	1.72	1.50	2.35
SD	±0.52	±0.51	±0.67				±0.82
p		ns[c]	ns	<.05	<.01	<.01	<.01
Dogs with residual hypophyseal function							
8	9.1 ± 1.7	3.7 ± 1.2	—	4.4	4.3	3.7	3.3
9	6.2 ± 1.8	3.8 ± 1.4	5.8	13.6	11.4	11.2	15.5
10[d]	2.2 ±0.4	10.2 (calcium)[a] ±3.6		2.9 (EDTA)[a] ±1.4			5.7

[a] 17-OH corticosteroid secretion rates in 3 consecutive 5-min collection periods measured (mean and SD). All other 17-OH corticosteroid secretion rates are single values.

[b] 17-OH corticosteroid secretion rates in the second, third, and fourth 5-min collection period after initiation of calcium administration.

[c] ns = not significant.

[d] Reversed order of administration; calcium preceded EDTA.

concentrations administered to the isolated adrenal pouch over a short period of time caused an acute increase in 17-OH CS secretion. However, no close correlation between the broad ranges of serum calcium concentrations observed and 17-OH CS secretion rates could be established ($r = 0.47$). The data suggest a pharmacological action of hypercalcemia to trigger an adrenal cellular mechanism resulting in increased 17-OH CS secretion. The precise location of calcium effect on steroid secretion is unknown, but some possibilities are: (i) the cell wall; (ii) the adenyl-cyclase-cyclic adenosine monophosphate system (cAMP); (iii) the mitochondria, to produce a swelling effect or to increase the 11β-hydroxylase enzyme activity; and (iv) vascular tree.

Although calcium is known to exert an important physiological effect on the cell membrane (9), a closer correlation between 17-OH CS secretion rates and calcium concentration might be expected if calcium were primarily active in the cell wall. On the other hand, an effect on cAMP is possible. Cyclic-AMP appears to underlie many hormonally stimulated events, (4, 10, 11). Calcium appears to be necessary for the cellular expression of cAMP generated effect, although a calcium effect on cAMP formation has not been excluded (2). Perhaps the hypercalcemia in the present stud-

ies augmented the effects of cAMP on steroidogenesis in some unknown way.

Another possible mechanism may involve induction of mitochondrial swelling with increasing calcium concentrations (12). Mitochondrial permeability to NADH (4) may increase, which may then furnish hydrogen to NADP to form NADPH, as catalyzed by mitochondrial transhydrogenase. Since NADPH is a stimulator of adrenal glucocorticoid secretion, calcium may operate by such a pathway. Finally, extensive isolated mitochondrial studies by Peron *et al.* (2, 3) have shown that the adrenal 11 β-hydroxylase activity is sensitive to changes in calcium concentration.

Although the data indicate a lack of consistent change in secretion of 17-OH CS after EDTA; nevertheless, in some experiments (Nos. 1, 5, 7, 8, 9) steep excursions of 17-OH CS secretion rates from the base line occurred. From these data alone, it is impossible to define the effect of transient hypocalcemia on CS secretion. Other possible influences, such as magnesium concentration were not measured; therefore, this cation, important in many biological systems (13), would have to be considered and controlled before the effect of EDTA-induced hypocalcemia on corticosterone secretion can be elucidated.

Since the arterial flow was kept constant and the venous effluent flow rates did not change with hypercalcemia, it is unlikely that calcium effect on 17-OH CS was mediated by a change in the adrenal vascular tree.

Summary. Wide variations in calcium concentrations were induced in arterial blood perfusing isolated dog adrenals. During NaEDTA, calcium fell without consistent change in 17-OH corticosteroid secretion. During calcium repletion and hypercalcemia secretion, rates of 17-OH corticosteroids rose significantly in both hypophysectomized and nonhypophysectomized dogs. Acute calcium administration, without prior Na EDTA, also produced increased 17-OH corticosteroid secretion. However, no close correlation between calcium concentrations and 17-OH corticosteroid secretion was observed. Hypercalcemia appears to stimulate glucocorticoid secretion in the whole adrenal gland *in situ*.

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