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Parathyroid Hormone Secretion in the Rat: Effect of Aminophylline (38536)

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It is well established that the bone (1, 2) and renal (3, 4) actions of parathyroid hormone (PTH) are mediated by adenosine 3′,5′-monophosphate (cyclic AMP). Previous *in vitro* studies by others (5) and from our laboratory (6) have shown that secretion of PTH is also mediated by cyclic AMP. In our *in vitro* studies, dibutyryl cyclic AMP, aminophylline and epinephrine all increased PTH release. However, aminophylline, which is known to increase cyclic AMP by inhibiting its destruction by phosphodiesterase (7, 8), was the most potent stimulus for PTH secretion.

The present study was undertaken to determine whether aminophylline can enhance PTH secretion in the intact animal.

Materials and Methods. Male albino Holtman rats weighing approximately 200 g were maintained on a nutritionally complete diet containing 1.7% calcium (Ca), 0.8% phosphorus, and 148 IU Vitamin D/100 g diet. Food was withheld for 16–20 hr before the experiments were initiated. Groups of rats received: (a) Normal saline, 0.5 ml im (control group), (b) aminophylline, 16 mg/kg body wt im, (c) disodium ethylene-diamine tetraacetic acid (EDTA) 150 mg/kg body wt ip, or (d) the same doses of both aminophylline and EDTA simultaneously. During the subsequent 2 hr, serial blood specimens were obtained by orbital sinus puncture. The serum was separated within 2 hr and frozen for subsequent analysis.

Serum Ca concentration was determined with the Technicon Auto Analyzer by the method of Kessler and Wolfman (9), or by the EDTA titration method of Hargis *et al.* (10). Serum immunoreactive PTH was determined by a sensitive radioimmunoassay technique for rat PTH developed in this laboratory (11).

The mean serial serum Ca and PTH concentration data for each group of rats were expressed in absolute values and also as a percent of the mean value of the control group at that time period. The group values were expressed as the mean ± SE and were statistically compared by Student's *t* test.

Results. After normal saline injection (control group), there was no significant change in either Ca or PTH during the following 2 hr (Table I). However, after EDTA injection, serum Ca decreased and PTH increased significantly ($P < 0.001$) by 0.5 hr (the earliest sampling time) (Table I). Both the Ca and PTH then gradually returned toward the control values over the next 1.5 hr of observation. Figure 1 shows this same data, with both the Ca and PTH concentrations plotted as percent of the control group values. After EDTA the serum Ca significantly decreased ($P < 0.001$) to 55.5 ± 3.8% of the control value and serum PTH significantly increased ($P < 0.001$) to 495 ± 44.1% of control by 0.5 hr after injection, with subsequent return toward the control values.

Table I and Fig. 1 illustrate the comparative effects of EDTA alone, aminophylline alone, or EDTA plus aminophylline on serum Ca and PTH. Aminophylline alone caused a significant rise ($P < 0.001$) in serum PTH to 211 ± 18.2% of the control value by 0.5 hr after injection, but there was no change in serum Ca concentration. EDTA plus aminophylline resulted in a decrease in serum Ca to the same degree as that after EDTA alone. At 0.5 hr after injection, the increase in PTH was the same after EDTA plus aminophylline as after EDTA alone. However, at 1 and at 2 hr after injection, the increases in PTH after EDTA plus aminophylline were to 782 ± 33.4% and 658 ± 28.8% of the control values, respectively. These increases are significantly greater than those after EDTA alone ($P < 0.001$) and also significantly greater than the sum of the

TABLE I. EFFECT OF INJECTION OF NORMAL SALINE, EDTA ALONE, AMINOPHYLLINE ALONE, OR EDTA PLUS AMINOPHYLLINE ON SERIAL SERUM Ca AND PTH CONCENTRATIONS DURING THE FOLLOWING 2 HR.

Substances injected	Substances analyzed	Time (h)			
		0	0.5	1	2
Normal	Ca[a]	9.3 ± 0.13	9.6 ± 0.13	9.4 ± 0.11	9.0 ± 0.10
Saline (Control)	PTH[b]	7.3 ± 0.25	6.4 ± 0.60	6.8 ± 0.49	7.6 ± 0.09
EDTA	Ca		5.2 ± 0.34[c]	6.3 ± 0.15[c]	7.1 ± 0.16[c]
	PTH		36.2 ± 0.26[c]	28.4 ± 1.1[c]	23.9 ± 1.8[c]
Aminophylline	Ca		9.2 ± 0.13	8.7 ± 0.36	8.7 ± 0.12
	PTH		15.3 ± 1.2[c]	15.4 ± 1.3[c]	15.1 ± 0.85[c]
Aminophylline plus EDTA	Ca		5.0 ± 0.20[c]	7.0 ± 0.02[c]	7.7 ± 0.28[c]
	PTH		32.9 ± 0.95[c]	57.2 ± 2.4[c]	48.1 ± 2.1[c]

Each value is the mean ± SE for six rats.

[a] Serum Ca expressed as mg/100 ml.

[b] Serum PTH expressed as picogram equivalents of bovine PTH/ml.

[c] Indicates values significantly different ($P < 0.001$) from mean values of the control group of rats at that time period.

separate PTH responses to EDTA and to aminophylline at 1 hr ($P < 0.01$) and at 2 hr ($P < 0.02$). The response to EDTA, to aminophylline and to EDTA plus aminophylline were also calculated as the area under the response curves from zero time until 2 hr after injection. Calculated in this manner, the PTH response to EDTA plus aminophylline was significantly greater ($P < 0.02$) than the sum of the separate PTH responses to EDTA and to aminophylline alone.

Discussion. These data indicate that the effect of aminophylline in enhancing PTH release is demonstrable *in vivo* as well as *in vitro* (6). Aminophylline (probably by inhibiting phosphodiesterase and hence increasing cyclic AMP) (7, 8), increased PTH secretion when given alone, and also enhanced the PTH secretion stimulated by EDTA-induced hypocalcemia. This enhancement was not discernible at 0.5 hr after injection, but was highly significant at 1 and 2 hr after injection, and in the total PTH response during the 2 hr observation period, actually exceeding the sum of the separate PTH responses to EDTA and to aminophylline. This lack of enhancement by aminophylline at 0.5 hr in spite of marked enhancement at 1 and 2 hr is unexplained by this study. A partial explanation may be that, at the later time periods, more of the hypocalcemia-induced cyclic

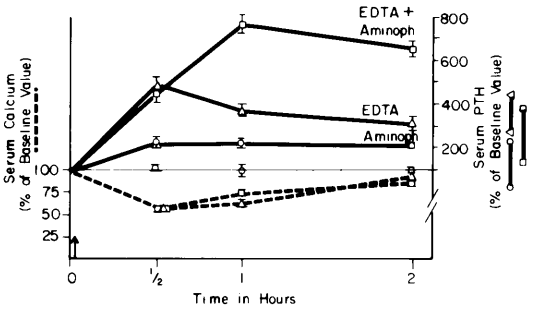

FIG. 1. Effect of injection of EDTA alone, aminophylline alone, or EDTA plus aminophylline on serial serum Ca and PTH concentrations during the following 2 hr. Both Ca and PTH are plotted as percent of the mean value of the control group of rats at that time period (indicated as 100%). Each point is the mean ± SE for six rats.

AMP was preserved by the aminophylline, resulting in greater cyclic AMP concentrations, and therefore greater PTH secretion. Further studies, including serial determinations of serum cyclic AMP and PTH concentrations simultaneously are needed to clarify this possibility. This aminophylline-augmented PTH secretion occurred without detectable changes in serum Ca.

The data indicate that PTH secretion *in vivo* (as well as *in vitro*) (6), can be increased by stimuli which enhance cyclic AMP. Fischer *et al.* (12) have recently shown that infusions of catecholamines [the beta adren-

ergic component of which stimulates cyclic AMP formation (13)] enhance PTH secretion *in vivo* in cows. Our studies further suggest that PTH secretion can be modified by influences other than those which act by modifying plasma Ca concentration. However, the data also support the concept that cyclic AMP is a mediator of low Ca-induced PTH secretion.

The dose of aminophylline per kg body wt administered in this study was within the therapeutic range for man. It is therefore possible that aminophylline may have an effect on PTH secretion in man; if so, long term aminophylline therapy may have important clinical implications in Ca homeostasis. Studies are currently underway to clarify this possibility.

Summary. Administration of aminophylline to intact rats did not cause a change in serum Ca, but did cause a significant increase in serum PTH. Administration of EDTA alone caused hypocalcemia, and a greater increase in PTH than that caused by aminophylline alone. Aminophylline plus EDTA given together caused no greater hypocalcemia, but a significantly greater increase in PTH than that caused by EDTA alone. Therefore, aminophylline increased PTH secretion when given alone, and also enhanced the PTH secretion stimulated by EDTA-induced hypocalcemia *in vivo*.

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