Parathyroid Control of Hypercalcemia Due to Injection of Parathyroid Extract in the Rat.* (28651)

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Although it has been generally agreed that the primary function of the parathyroids is to control hypocalcemia, Sanderson *et al*(1) observed that the hypercalcemia induced by calcium infusion was not controlled as effectively in parathyroidectomized dogs, and Tweedy and Chandler(2) noted that parathyroidectomized rats appeared to be more sensitive than normals to the hypercalcemic effect of injected parathyroid extract. The recent discovery of calcitonin(3), a hypocalcemic hormone from the parathyroids, has suggested that these glands may indeed play an active role in controlling hypercalcemia. Such a control appears to be operative in the dog following stimulation of endogenous parathormone output by EDTA induced hypocalcemia(4) or infusion of parathyroid extract (5). The following experiments were carried out to determine whether the parathyroids exerted a similar control in the rat.

In developing a biological assay for parathormone, Munson(6) observed that when rats fed a low calcium diet were parathyroidectomized and injected with parathyroid extract, the plasma calcium level 6 hours later was directly related to the logarithm of the dose injected. Extrapolation of his data suggests that a dose of approximately 83 u PTE (parathyroid extract, Lilly) per 100 g injected immediately after parathyroidectomy would be expected to elevate the plasma calcium 2 mg–% 6 hours later. This was compared to the response to the same dose in animals with intact parathyroids.

Methods. In the first series, 6-week-old rats maintained on stock diet were divided at random into 2 groups. A blood sample (1 ml) was drawn by heart puncture, and the thyroid and parathyroid glands were removed surgically in the first group, while a sham operation was performed in the others. Each animal received 100 u PTE (Para-Thor-Mone, Lilly) by subcutaneous injection, the average dose being 85.8 ± 1.6 u/100 g. Six hours later, a second blood sample was drawn by heart puncture, and the plasma calcium was determined by EDTA titration, using the method of Copp(7).

Older animals (2 months) were used in the second series, and only the parathyroids were removed surgically, leaving the thyroid glands intact. The dose of PTE injected subcutaneously was adjusted in each animal to 85 u/100 g. In the second group, the same dose was administered to animals with intact parathyroids. A control group with intact glands was injected with an equivalent volume (0.85 ml/100 g) of saline.

Results. The changes in plasma calcium in the first series are shown in Fig. 1 and summarized in Table I. Although injection of PTE caused a significant rise in plasma calcium in both groups, the increase in the thyroparathyroidectomized group was over 3 times as great, and plasma calcium level was significantly higher than in the animals with intact glands (Difference = 1.364 ± 0.266 mg–%, $t = 5.12$, $p < 0.001$).

Similar results were obtained in the second

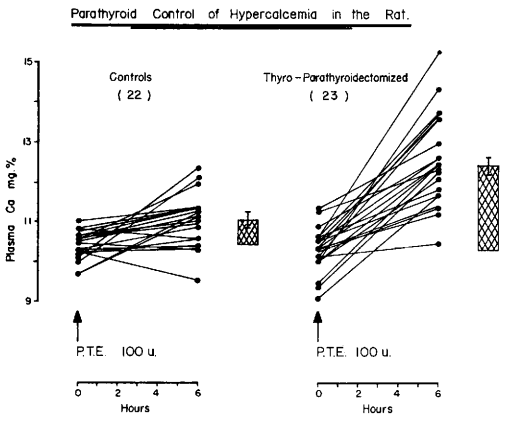

FIG. 1. Change in plasma calcium after subcutaneous injection of 100 u parathyroid extract into thyro-parathyroidectomized and sham operated control rats.

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TABLE I. Effect of Thyro-parathyroidectomy on Response of Plasma Ca to Injected Parathyroid Extract.*

Group	No.	Plasma calcium (mg %) Initial (P_0)	After 6 hr (P_6)	Difference (Δ)	t	p
Sham operated controls (parathyroids intact)	29	10.372 ±.071	10.934 ±.115	+ .562 ±.121	4.64	<.001
Thyro-parathyroidectomized	30	10.292 ±.089	12.298 ±.241	+1.971 ±.224	8.80	<.001

* Values expressed as average ± standard error of mean.
t = Fisher test ratio = Δ/s.e. p = probability that difference is due to chance.

TABLE II. Effect of Surgical Parathyroidectomy on Response of Plasma Ca to Injected Parathyroid Extract.*

Group	No. of rats	Plasma calcium (mg %) Initial (P_0)	After 6 hr (P_6)	Difference (Δ)	t	p
1. Saline injected controls (parathyroids intact)	17	9.420 ±.105	9.463 ±.099	+ .043 ±.144	.30	n.s.
2. P. T. E. injected						
(a) parathyroids intact	18	9.456 ±.127	9.757 ±.074	+ .307 ±.147	2.10	<.05
(b) parathyroidectomized	24	9.303 ±.064	11.278 ±.277	+1.975 ±.283	6.98	<.001

* Values expressed as average ± standard error of mean.
t = Fisher test ratio = Δ/s.e. p = probability that difference is due to chance.

series in which only the parathyroid glands were removed (Table II). The lower initial plasma Ca may be attributed to the older age of these animals. There was no significant effect of saline injection but there was a significant rise following PTE injection. This rise was over 6 times as great in the parathyroidectomized animals, despite the presence of intact thyroids, and there was a highly significant difference in the 6 hour plasma level (Difference = 1.521 ± 0.286 mg – %, t = 5.30, p < 0.001).

Discussion. The hypercalcemic response to subcutaneous injection of 85 u PTE/100 g into parathyroidectomized rats was similar to that predicted from extrapolation of the data of Munson(6), whether the thyroid glands were removed simultaneously or were left intact. However, this response was significantly reduced in the animals with intact parathyroids, indicating that these glands exert a controlling effect on PTE induced hypercalcemia in the rat, just as they do in the dog (5). Demole and Fromherz(8) observed that the hypercalcemia produced in rats by toxic doses of Vit. D_2 was enhanced by parathyroidectomy, and Stoerk and Celozzi(9) have recently reported that removal of these glands increased the severity of calcinosis produced by PTE injection into rats. The control is apparently due to the parathyroids rather than the thyroids, and may be explained by liberation of the hypocalcemic hormone, calcitonin, in response to hypercalcemia. It has been suggested that a rise of less than 0.5 mg – % in plasma calcium in the dog may provide an adequate stimulus for release of this hormone(10), and it is of interest that this is approximately the increase observed in the PTE injected rats with intact parathyroids.

Summary and conclusions. Following subcutaneous injection of 85 u parathyroid extract/100 g into young rats, the rise in plasma calcium 6 hours later was 3-6 times as great when the parathyroids were removed as in the control rats with intact glands. The smaller response in the latter was attributed to a controlling effect of the parathyroids on hypercalcemia through liberation of the hypocalcemic hormone, calcitonin.

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Effect of Aldosterone on Ouabain-Induced Potassium Loss from Isolated Rabbit Atria.* (28652)

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Aldosterone has been shown to stimulate or enhance active sodium (Na^+)-potassium (K^+) transport in a variety of tissues both *in vivo* and *in vitro* (Gross, 1,2). On the other hand, the cardiac glycoside ouabain is recognized as a potent inhibitor of active Na-K transport across cell membranes(3), including heart muscle(4). Based on these results, therefore, a possibility exists that these 2 steroids might be mutually antagonistic insofar as their cation transport effects are concerned. Although various studies have indicated that aldosterone (as well as other corticosteroids) is ineffective in antagonizing cardiac glycoside-induced changes in cation transport in the red blood cell (RBC)(5-7), there is some evidence that aldosterone may be able to prevent the K^+ loss from isolated hearts produced with high doses of cardiac glycosides(8,9), as well as antagonize the effects of ouabain on active Na^+ transport in isolated frog skin when the steroids are given *in vivo*(10).

This conflicting evidence on the alleged ability of aldosterone to block or modify the actions of ouabain on cation transport has prompted us to investigate whether or not this naturally occurring hormone can, in fact, prevent or modify the changes in K^+ content induced with high concentrations of ouabain in stimulated isolated heart muscle. We have previously shown that insofar as the electrical and contractile parameters of isolated atrial tissue are concerned, d,l-aldosterone, in equimolar concentration, does not antagonize the actions of ouabain(11), with the apparent exception of the contracture response. In a preliminary study, we were unable to demonstrate an antagonism of ouabain-induced K^+ loss using 1×10^{-6}M aldosterone(12). The present report extends these observations and includes the effects of higher (2.2×10^{-6}M-10^{-5}M) concentrations of d-aldosterone on the K^+-depleting effect of ouabain.

Methods. Experiments were performed on electrically driven, isolated rabbit left atrial preparations. Tissue was obtained from hearts of albino rabbits weighing 1.5-2.5 kg. The general procedures for dissecting and preparing the muscle were the same as outlined previously(13,14). The left atria used were free of any pacemaker tissue, and any preparation which showed spontaneous beating during the dissection or mounting procedure was discarded. The tissue was suspended horizontally in a 30 ml muscle chamber perfused with a Ringer solution of the following composition (mM/l); NaCl, 154; KCl, 5.6; $NaHCO_3$, 5.9; CaCl, 2.2; and dextrose, 5.5. The perfusate was gassed with a 95% O_2 – 5% CO_2 gas mixture via a sintered disc placed directly in the muscle chamber. All experiments were performed at 30°C. The

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