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Effect of Long-Term Administration of Epinephrine and Propranolol on Serum Calcium, Parathyroid Hormone, and Calcitonin in the Rat (40329)

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Previous short-term *in vitro* and *in vivo* studies have shown the importance of β-adrenergic stimuli in the secretion of parathyroid hormone (PTH) (1–5) and calcitonin (CT) (6–8). In these studies, β-adrenergic agonists, epinephrine and isoproterenol, increased PTH and CT secretion, whereas the β-adrenergic antagonist, propranolol, inhibited the secretion of these two hormones. Subsequent studies have suggested that the effects of isoproterenol in the perifusion system (9) and of epinephrine *in vivo* in the cow (10) may be short-lived, lasting for 40 to 50 min. The present studies therefore evaluated the effects of long-term administration of epinephrine or propranolol on PTH and CT secretion in the rat.

Materials and methods. Sprague–Dawley rats weighing 250 to 300 g were divided into three groups.

Group I rats received daily im injection of l-epinephrine in sesame seed oil (0.3 mg/day for 2 weeks followed by 0.6 mg/day for an additional 3 weeks) ($n = 5$).

Group II rats received *dl*-propranolol[1] (approximately 40 mg/day) for 5 weeks in their drinking water and in addition received daily im injections of sesame seed oil ($n = 4$).

Group III rats served as control and received daily im injections of sesame seed oil ($n = 6$).

All animals were bled via orbital sinus puncture at weekly intervals with bleedings being performed 24 hr after the last injection. Serum was separated within 2 hr of the bleeding and frozen for subsequent analysis for serum PTH, CT, calcium, and total proteins.

Serum parathyroid hormone was determined by a slight modification of the previously described method for rat PTH developed in our laboratory (11). The present method utilizes an antibody against bovine parathyroid hormone developed in a goat. Figure 1 illustrates a standard curve prepared with the use of this antiserum in the dilution of 1:20,000, ^{131}I-labeled bovine PTH, and various concentrations of unlabeled bovine PTH; the antibody bound (B)/free(F) values are expressed as a percentage of initial or trace B/F. Figure 1 also shows the percentage B/F values when (a) increasing volumes (50–200 μl) of serum from a rat, obtained 48 hr after bilateral nephrectomy, and (b) increasing volumes (1–20 μl) of pooled tissue culture medium, in which rat parathyroid glands were cultured for 48 hr, were added. It is apparent that the displacement curves for PTH in bovine standard, rat serum, and tissue culture medium from rat parathyroid glands are superimposable. In addition, by utilizing this antiserum, appropriate changes are observed (data not shown) in serum PTH by induced hypo- or hypercalcemia in the rat. Parathyroidectomized rats demonstrate undetectable serum levels of PTH. Basal serum PTH levels in the normal rats with this assay are 4.27 to 6.35 pg-equiv of bovine PTH/ml ($n = 22$).

Serum calcitonin was determined by a method similar to the one developed in our laboratory for human and monkey CT (12). The assay utilizes an antibody developed against human synthetic CT in a goat. Human CT is also used as the tracer and the standard. Figure 2 illustrates a standard curve prepared with the use of this antiserum in the dilution of 1:20,000, ^{131}I-labeled human CT, and various concentrations of unlabeled human CT; the B/F values are expressed as a percentage of initial or trace B/F. Figure 2 also shows the percentage B/F values when

[1] *dl*-Propranolol was kindly supplied by the Ayrest Laboratories, New York, New York. Fifty milligrams was dissolved in 50 ml of water and kept in light-proof drinking water bottles. Each rat consumed approximately 40 ml of water daily.

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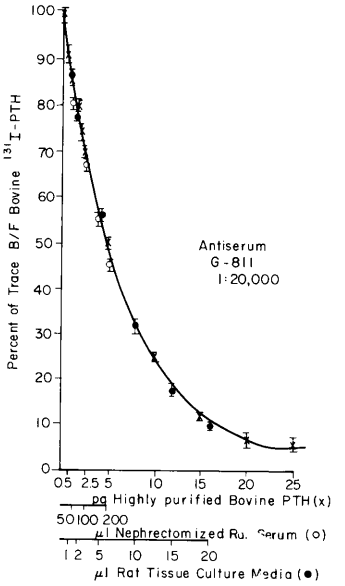

FIG. 1. Comparison of tracer displacement curves for highly purified bovine PTH, serum from a nephrectomized rat, and tissue culture medium from rat parathyroid glands. B/F values along the ordinate are expressed as a percentage of the initial or trace B/F. Concentration scales along the abscissa are adjusted as shown to allow superimposition of one point of each curve to allow determination of similarity of curves. Each point represents the mean ± SD of six replicates in a single assay.

(a) increasing volumes (1–70 μl) of plasma from a calcium-infused rat and (b) increasing volumes (10–200 μl) of an acetone–acetic acid extract of thyroid gland from a rat were added. It is apparent that the displacement curves for CT in human CT standard, rat plasma, and rat thyroid extract are superimposable. Basal serum CT levels in the normal rat with this assay are 134 to 231 pg-equiv of human CT/ml (n = 22) and there is a 2- to 8-fold increase in this value with calcium infusion (n = 8). The levels of CT become undetectable following thyroidectomy. Intra-assay coefficient of variation with this assay for the normal pooled rat serum is 3.5%. All samples for parathyroid hormone and for calcitonin were analyzed in single assays.

Serum calcium was determined by the EGTA titration method (13). Serum total proteins were determined by refractometry (American Optical Corp., Buffalo, N.Y.).

Group mean values for the experimental groups for a given time period were compared with those of the control group by Student's t test.

Results. The animals tolerated the injection procedures and propranolol administrations well and gained weight normally. Initial weights were 255 ± 4, 254 ± 5, and 254 ± 3 and the final weights at the end of the study were 334 ± 2, 334 ± 3, and 327 ± 6 g for groups I, II, and III respectively.

Figures 3 and 4 depict the changes in serum PTH and CT, respectively, in the rats receiving epinephrine, propranolol, or vehicle. There were no significant changes observed with time in either the serum PTH or CT levels in the vehicle-injected control rats. The concentrations of both serum PTH and CT were significantly increased in epinephrine-injected rats as compared to control animals at the end of 2 and 3 weeks, respectively, with further progressive increases during the remainder of the study. The maximum concentrations of PTH and CT were 158 ± 8 and 173 ± 25% of control, respectively, and were reached at the end of 5 weeks.

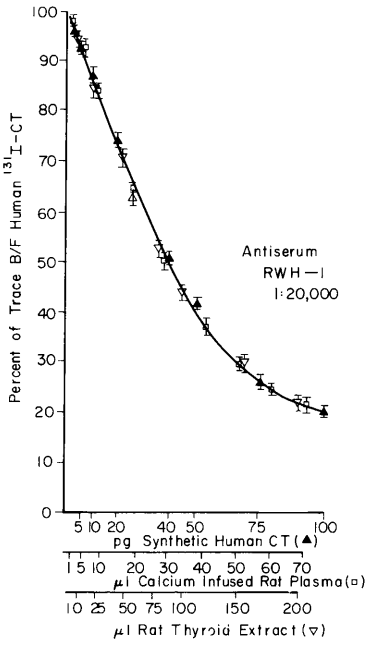

FIG. 2. Comparison of tracer displacement curves of synthetic human CT standard, serum from a calcium infused rat, and acetone acetic acid extract of a rat thyroid gland. B/F values along the ordinate are expressed as a percentage of the initial or trace B/F. Concentration scales along the abscissa are adjusted as shown to allow superimposition of one point of each curve to allow determination of similarity of curves. Each point represents the mean ± SD of six replicates in a single assay.

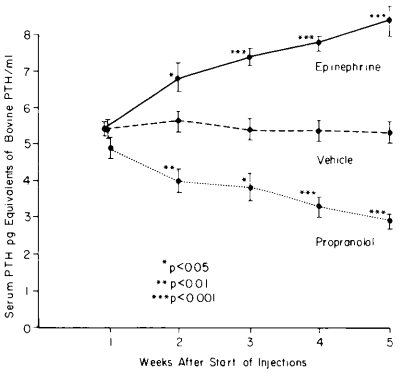

FIG. 3. Effect of administration of epinephrine, propranolol, or vehicle on serum parathyroid hormone concentration. Each point represents the mean ± SE. The data are expressed in absolute values. See text for percentage changes.

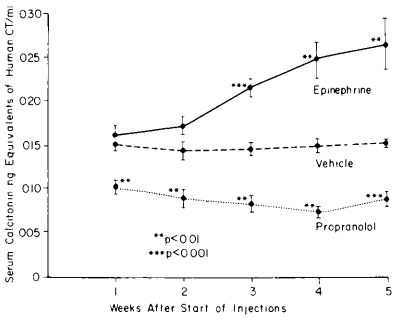

FIG. 4. Effect of administration of epinephrine, propranolol, or vehicle on serum calcitonin concentration. Each point represents the mean ± SE. The data are expressed in absolute values. See text for percentage changes.

The concentrations of both serum CT and PTH were significantly decreased in rats receiving propranolol as compared to control animals at the end of 1 and 2 weeks, respectively, with further progressive decreases during the remainder of the study. The lowest concentrations for serum CT and PTH were 49 ± 4 and 54 ± 5% of control and were reached at the end of 4 and 5 weeks, respectively.

Figure 5 demonstrates that serum calcium values were not significantly different among the three groups at any time tested during the study.

Serum total proteins did not significantly change during the study in any of the groups.

Discussion. β-Adrenergic stimuli have been

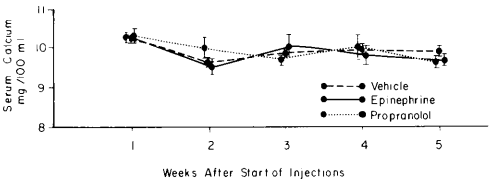

FIG. 5. Effect of administration of epinephrine, propranolol, or vehicle on serum calcium concentration. Each point represents the mean ± SE.

shown to play a role in the secretion of PTH (1–5) and CT (6–8) in short-term studies. The present studies clearly demonstrate that long-term modification of β-adrenergic stimuli by administration of large doses of epinephrine and propranolol can also affect serum concentration of these two hormones. In the previous short-term studies, the stimulatory effects of isoproterenol and epinephrine on PTH and CT have been shown to be β-adrenergic as these could be blocked by propranolol (1, 4, 6).

The changes in the serum PTH and CT observed in the present studies were not due to hemoconcentration or hemodilution, as there was no change observed in the serum protein concentration. The present studies do not entirely exclude the possibility that the changes observed in serum PTH and CT were not due to changes in their peripheral metabolism. However, epinephrine and propranolol can respectively stimulate or inhibit PTH secretion in *in vitro* studies (1). Therefore, it is likely that the changes observed in the serum concentrations of PTH in the present study were because of changes in its secretion. The changes observed in the serum concentration of CT were also presumably because of changes in its secretion.

The lack of change in serum calcium observed in the present studies may possibly be explained on the basis of simultaneous comparable changes in both the PTH and CT which have opposite effects on serum calcium concentration.

Previous case reports (14, 15) of two patients with pheochromocytoma and evidence of excessive PTH production, one of whom had hypercalcemia (14), suggested that long-term excess of catecholamines may cause hyperparathyroidism. However, in subsequent studies, serum PTH levels were found to be normal in 10 unselected patients with pheo-

chromocytoma (16). The present studies show that, at least in the rat, long-term excess of catecholamines can increase serum PTH concentrations.

Summary. Injection of epinephrine to 250- to 300-g rats (0.3 mg/day for 2 weeks, followed by 0.6 mg/day for another 3 weeks) progressively increased the serum PTH and CT, whereas administration of approximately 40 mg of propranolol daily, in drinking water, progressively decreased the serum levels of both these hormones in comparison to control animals. The studies indicate that, similar to the short-term effects observed in previous studies, long-term modification of β-adrenergic stimuli can affect PTH and CT secretions.

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