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Corticosteroidogenesis *in Vitro*: Effects of Parathyroid Hormone, ACTH, and Calcium[1] (41187)

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Abstract. Isolated rat adrenocortical cells were incubated with bovine parathyroid hormone (PTH) in the absence and presence of ACTH and $CaCl_2$. PTH had no effect on corticosterone production, but $CaCl_2$ induced steroidogenesis in the absence or presence of ACTH. Maximal corticosterone production in the absence of ACTH occurred with 1.25 mM $CaCl_2$ and in the presence of ACTH with 5.00 mM $CaCl_2$. These results show that PTH does not directly affect acute corticosterone production by isolated adrenocortical cells. They also show that ACTH raises the maximal steroidogenesis inducible by $CaCl_2$.

The mechanism by which parathyroid hormone (PTH) increases the circulating level of corticosterone and causes adrenal hyperplasia (1) is not clear. Several investigators favor the view that PTH acts *in vivo* indirectly by increasing the level of serum calcium which in turn increases glucocorticoid secretion (1–4). However a direct action of PTH on adrenocortical cells is also compatible with these findings. PTH does have direct effects on other cells, such as thymic lymphocytes (5), fibroblasts (6) and renal (7–11), skeletal (8–11), and hepatic cells (11); however, it is not known whether or not PTH directly affects adrenocortical cells.

To address this question we have studied these cells *in vitro*. We have used isolated adrenocortical cells to determine whether PTH alone or in combination with Ca^{2+} and ACTH directly affects corticosterone production. These *in vitro* studies were designed to eliminate any possible systemic changes produced by PTH or Ca^{2+} infusion *in vivo* that could secondarily affect adrenocortical function and complicate the interpretation of the results.

Materials and Methods. Male Sprague–Dawley rats (300–400 g) were maintained on a 14 hr per day light cycle. They were fed Purina Formulab No. 5008 and water *ad libitum*. Adrenocortical cells were isolated as described by Sayers *et al.* (12). The steroidogenic properties and the fine structure of these cells have been described (13). The basic cell incubation medium was Krebs–Ringer bicarbonate buffer (7.62 mM $CaCl_2$) with 0.2% glucose, 0.5% bovine serum albumin, and 0.1% lima bean trypsin inhibitor. Aliquots (1.0 ml) of the isolated cell suspensions (25,000 cells/ml) were incubated in plastic culture tubes (12 × 75 mm) in a Dubnoff shaker (66 oscillations/min) at 37° under an atmosphere of 95% O_2–5% CO_2 for 2 hr and then frozen until assayed for corticosterone. In each experiment at least 87% of the cells were viable after incubation as indicated by trypan blue exclusion (14).

Varying concentrations of ACTH-α-1-24 (Cortrosyn) and both active and inactive preparations of bovine parathyroid hormone (1–34 and 1–84 peptides) and $CaCl_2$ were added to the basic cell medium. Bovine PTH (1–84) and synthetic bovine PTH (1–34) were prepared by methods previously described (15, 16). Inactivation by oxidation of both preparations was achieved by addition of 1.0 ml of performic acid to tubes containing 0.1 μM peptide (17). After incubation at 0° for 2 hr, the samples were diluted with 5 vol of cold dis-

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tilled water and then lyophilized. When measured by the rat renal adenylate cyclase assay (18), the bioactivity of preparations of bovine PTH (1–84) and bovine PTH (1–34) was 3000 and 5400 MRC (Medical Research Council) units/mg, respectively. The bioactivity of neither oxidized preparation was greater than 2% of that of the untreated bovine PTH standard.

In the experiments in which the $CaCl_2$ concentration of the incubation medium was varied, a trypsin solution containing 1.25 mM $CaCl_2$ was used for the cell isolation prior to the incubation. This $CaCl_2$ concentration was lower than that (2.54 mM) used routinely for cell isolation in our other experiments. It is within the normal range (1.1–1.3 mM) of ionized plasma calcium concentration (19).

Corticosterone, which is the major glucocorticoid secreted by the rat adrenal cortex (20), was measured by a modification (G. J. Macdonald, personal communication) of the radioimmunoassay (RIA) procedure of Roy *et al.* (21) using corticosterone as the standard. The cortisol antibody used in the RIA cross-reacts completely with corticosterone (21) and thus the data are expressed as nanograms corticosterone per milliliter. Analysis of variance (single classification, samples within samples, Model II) (22) was used to evaluate the data; means were deemed significantly different when $P \leq 0.05$.

Results. Active or inactive bovine PTH-1–34 over a wide range of concentrations (10^2 to 10^5 pg/ml) did not alter the corticosterone production of isolated rat adrenocortical cells in the absence of ACTH or in the presence of half-maximal (6.25 pg/ml) or maximal (100 pg/ml) steroidogenic concentrations of ACTH ($P \geq 0.10$) (Fig. 1). The concentration of $CaCl_2$ was kept constant at 7.62 mM.

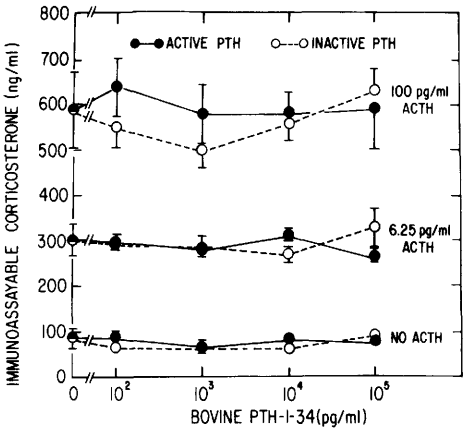

FIG. 1. Ineffectiveness of bovine parathyroid hormone (PTH-1-34) on corticosterone production of isolated adrenocortical cells of the rat in the absence and presence of ACTH. $CaCl_2$ was constant at a concentration of 7.62 mM. Cell suspensions were incubated for 2 hr. Each symbol represents the mean corticosterone values from nine cell suspensions (three suspensions from each of three experiments). Standard errors are represented by bars when they are larger than the symbols.

Cells incubated in various combinations

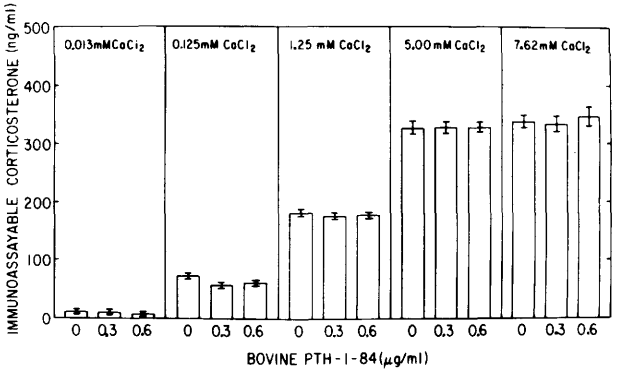

FIG. 2. Effect of calcium on corticosterone production of isolated adrenocortical cells of the rat incubated with ACTH (6.25 pg/ml) in the absence or presence of bovine parathyroid hormone (PTH-1–84). Cell suspensions were incubated for 2 hr. Each column represents the mean corticosterone values from nine cell suspensions (three suspensions from each of three experiments). Standard errors are represented by bars.

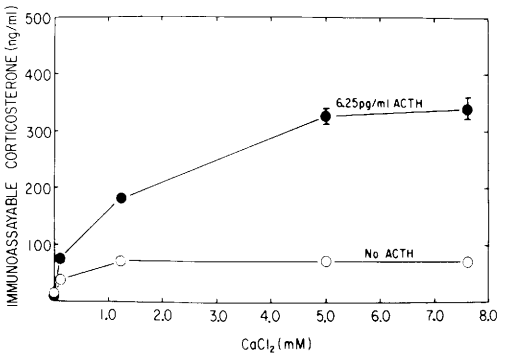

FIG. 3. Effect of calcium on corticosterone production of isolated adrenocortical cells of the rat in the absence and presence of ACTH. Cell suspensions were incubated for 2 hr. Each symbol represents the mean corticosterone values from nine cell suspensions (three suspensions from each of three experiments). Standard errors are not shown because they are smaller than the symbols.

of PTH peptides and ACTH (6.25 pg/ml) (Figs. 2 and 3) did not stimulate steroidogenesis when the $CaCl_2$ concentration was as low as 0.013 mM. However, $CaCl_2$ at concentrations of 0.125–7.62 mM promoted steroidogenesis in the absence (Fig. 3) or presence (Figs. 2 and 3), of ACTH. In the absence of ACTH, steroid production could not be increased by raising the $CaCl_2$ concentration above 1.25 mM (Fig. 3). However, in the presence of ACTH, steroid production was increased by $CaCl_2$ up to a concentration of 5.00 mM $CaCl_2$ (Figs. 2 and 3). Maximal steroid production in the presence of ACTH was about four times greater than in its absence. Again, active preparations of bovine PTH-1-84 (0.3 or 0.6 μg/ml) did not alter corticosterone production regardless of the presence of ACTH or $CaCl_2$ ($P \geq 0.10$) (Fig. 2). Similarly, inactive preparations of bovine PTH-1-84 (0.3 or 0.6 μg/ml) did not alter corticosterone production regardless of the presence of ACTH or $CaCl_2$ (data not shown).

Discussion. The existence of a parathyroid–adrenal relationship was first established in 1911 when it was demonstrated that removal of the adrenal glands prevented tetany in parathyroidectomized animals (23). This finding suggested that the adrenal glands produced a factor which lowered the level of serum calcium. Other studies also showed that glucocorticoids in many instances prevented or ameliorated certain actions of parathyroid hormones on calcium homeostasis (1, 24–28). Paradoxically, removal of the adrenal glands also antagonized some actions of PTH (29).

In addition to these reports indicating that the adrenal hormones can modulate the actions of parathyroid hormone, there is evidence that PTH can affect the adrenal cortex, producing hyperplasia and raising plasma glucocorticoids (1). PTH affects thymic (5) and hepatic (30) cells by stimulating production of cAMP, a compound which increases glucocorticoid production of adrenocortical cells (31–35). Thus the possibility existed that PTH action on adrenocortical cells might be a direct one involving cAMP levels. However, we found that PTH did not significantly alter ($P \geq 0.10$) corticosterone production of these cells either in the absence (Fig. 1) or presence (Fig. 2) of ACTH.

An increase in intracellular calcium would provide another means whereby PTH might directly stimulate corticosteroidogenesis. Calcium is necessary for maximal corticosteroidogenesis induced by ACTH (36, 37–41), cAMP (42), or guanosine 3′,5′-monophosphate (40). PTH promotes calcium influx in a variety of cell types *in vitro* (43–47) as well as calcium exchange *in vivo* (48). In our experiments (Fig. 2), however, PTH failed to alter ACTH-induced corticosterone production over a large range of $CaCl_2$ concentrations ($P \geq 0.10$).

In several instances ACTH and $CaCl_2$ were used at concentrations that were submaximal for corticosterone production (0.013, 0.125, and 1.25 mM $CaCl_2$; 6.25 pg/ml ACTH) (Figs. 2 and 3). Also, some of the $CaCl_2$ concentrations were below the normal range of ionized plasma calcium concentrations (0.013 and 0.125 mM $CaCl_2$) (Figs. 2 and 3)). Thus, the ineffectiveness of PTH was not due to the fact that the cells were already maximally stimulated.

Although our results show that PTH does not affect corticosterone production by isolated adrenocortical cells during a 2-hr incubation period, a long-term direct action

of PTH on the adrenal cortex cannot be ruled out. On the other hand, *in vitro* studies suggest that the action of PTH is indirect and may be the result of hypercalcemia (1). Indeed, hypercalcemia induced either by calcium infusion (2) or by administration of PTH (3) increased glucocorticoid secretion.

The data in Fig. 2 show that steroidogenesis varied directly with calcium concentration in the presence of ACTH and are in agreement with other work *in vivo* (2, 3) and *in vitro* (36, 40, 41, 49, 50). Figure 3 shows that this calcium-induced corticosterone production also occurs in the absence of ACTH and with one exception (40) is consistent with a number of reports (38, 51–53). However, it is obvious that the maximal level of corticosterone production that Ca^{2+} can induce is about four times greater in the presence of ACTH than in the absence of ACTH. These data extend early reports (12, 36, 40, 51) and clearly indicate that ACTH enhances the maximal level of steroidogenesis that can be stimulated by Ca^{2+} as well as vice versa.

Thus PTH might increase corticosteroidogenesis indirectly *in vivo* by increasing the serum Ca^{2+} available to the adrenocortical cell. This action of PTH would be enhanced by ACTH either by aiding the binding (54, 55), translocation (50), or entry (56–59) of Ca^{2+} into the adrenocortical cell. Whatever the mechanism, the view proposed here is that ACTH permits the adrenocortical cell to respond to higher concentrations of Ca^{2+} than is possible in the absence of ACTH.

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