Hormonal Effects on Cyclic AMP in a Renal-Cell Suspension System[1] (34977)

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Since the discovery of cyclic 3′, 5′-adenosine monophosphate (cyclic AMP) it has been recognized that several hormones exert at least some of their effects by causing an alteration in the intracellular level of this nucleotide. Some of these hormones are glucagon, adrenocorticotropin, vasopressin, luteinizing hormone, and catecholamines (1). That cyclic AMP may play a role in the action of parathyroid hormone has been suggested by Chase and Aurbach (2) who have found that this hormone caused an increase in urinary excretion of cyclic AMP.

In a previous communication in this journal (3) we have reported the preparation of a dog renal-cell suspension which was found to be suitable for the study of renin production *in vitro*. This cell-suspension system has the advantage that it can be used to study renal-cell physiology in the absence of neurogenic or hemodynamic influences. A renal slice preparation might have the difficulty that relatively few of its cells are in direct contact with the incubation medium. While cell-free homogenates of renal tissue overcome this difficulty, they are obviously unsuitable for studies of metabolic functions which depend upon cellular integrity. The renal-cell-suspension system might be a versatile tool in studies of renal metabolism because it combines the advantages of cellular integrity with those of exposure of cell surfaces. We utilized this cell-suspension system to study the effects of vasopressin and parathyroid (PTH) hormone on the cyclic AMP content of the renal cells. We found that both hormones stimulate its formation.

Although the observed rise in cyclic AMP in the incubated renal-cell suspensions is not a direct evidence of the mechanism of action of these hormones, it supports the view that it may play a role in the physiological reponse of the kidney to these polypeptides.

Materials and Methods. Materials: The materials used were parathyroid hormone USP, product of Eli Lilly Co., Indianapolis, Indiana; pitressin (Vasopressin), product of Parke, Davis & Co., Detroit, Michigan; medium (3); and collagenase, product of Nutritional Biochemicals Corp., Cleveland, Ohio.

Methods. The renal-cell suspension was prepared as described in detail in a previous report (3). In brief, young mongrel dogs were anesthetized with pentobarbital, and the kidneys were excised. After washing each kidney with physiological saline followed by perfusion with 0.3% collagenase in the medium, the renal cortex was removed and minced. These fragments were then digested in the collagenase solution for 1 hr. The cells obtained were separated and washed to remove adherent collagenase and then resuspended in the medium and within 30 min were used in the experimental incubations as described below. Microscopic examination of each renal cortical cell suspension revealed intact cells. Some tubular fragments were found, but intact glomeruli were not identified.

The cyclic AMP in the cell-suspension samples was assayed by a procedure previously described (4, 5).

Experimental incubations. In each experiment, 5-ml aliquots of the well-mixed suspen-

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sion were added to each flask. Cell breakage during transfer was minimized by using pipettes with wide tips. All samples were run in duplicate. In each experiment, two samples were not incubated but were processed for measurement of cyclic AMP levels to be used as nonincubated controls. In some experiments two of the nonincubated samples were homogenized (4, 5) and parathyroid hormone was added prior to the cyclic AMP determination. These samples were used to determine whether parathyroid hormone interferes with the cyclic AMP assay. To series of duplicate samples parathyroid hormone was added and then they were incubated in a 37° water bath with slow constant shaking under an atmosphere of 95% oxygen and 5% carbon dioxide. The period of incubation ranged from 5–20 min. At the end of each incubation period the samples were homogenized and processed for measurement of their cyclic AMP content (4, 5). Under identical conditions, duplicate samples without parathyroid hormone were incubated and then processed for measurement of cyclic AMP levels to be used as incubated controls.

Results. Effect of PTH on cyclic AMP concentration in isolated renal cortical cells. In all experiments, the addition of PTH to the medium caused a marked rise in renal-cell cyclic AMP concentration, whereas the cyclic AMP concentration in the samples incubated without PTH remained unchanged. These results with renal-cell suspension agree qualitatively with those reported by other investigators who performed experiments with other preparations (2, 6). The results of two representative experiments with different cell-suspension samples are shown in Fig. 1. The highest concentration of cyclic AMP was obtained at the 10-min incubation period.

Studies were also performed to find out whether the observed rise in cyclic AMP with PTH was due to the interference of this hormone with the procedure of measuring cyclic AMP. Along with the experimental samples, a total of seven pairs of duplicate samples were incubated without PTH. At the end of the incubation these samples were homogenized and then PTH was added. Whereas the cyclic AMP content, in the samples in which PTH was added prior to the incubation, rose 4- to 10-fold, there was no change in cyclic AMP content in those samples which were incubated without PTH, and this hormone was added after the homogenization step of the cyclic AMP assay procedure.

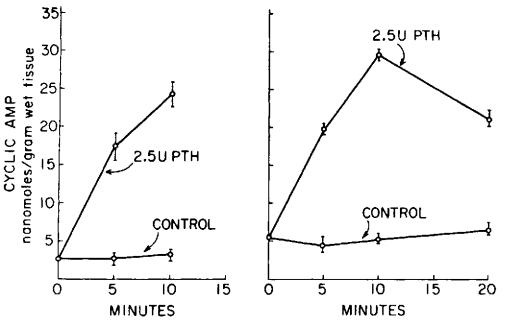

FIG. 1. Time course of the effect of PTH on the cyclic AMP concentration in renal cortical cell suspensions. The lower curve represents samples incubated without PTH. The upper curve represents samples incubated with PTH 2.5 units per ml of incubation medium. Brackcted vertical lines indicate SD.

Effect of pitressin on cyclic AMP concentration in isolated renal cortical cells. The response of cyclic AMP content to pitressin was tested utilizing the renal-cell-suspension system. As in the case of PTH, duplicate samples of the cell suspension were incubated with and without pitressin for periods of 10–20 min, and the cyclic AMP concentration was determined. In all experiments there was a rise in cyclic AMP with the addition of pitressin but to a lesser extent than that obtained with PTH. The results of a representative experiment was shown in Fig. 2.

The response of cyclic AMP concentration to various doses of stimulants. The effects of different concentrations of PTH and pitressin on the cyclic AMP levels of the renal-cell suspensions are shown in Fig. 2. The larger the dose of hormone added the higher the concentration of cyclic AMP in the incubated cell-suspension samples. Although various doses were used, the rise of cyclic AMP concentration was greater with PTH than with pitressin.

The same results in the various series of

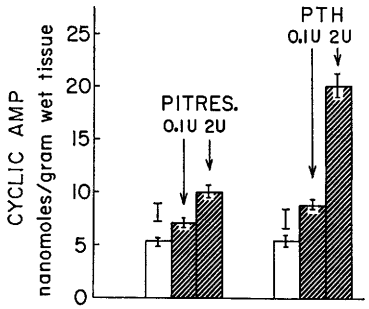

FIG. 2. The response of cyclic AMP to various stimulants: I = incubated control suspensions; PITRES = pitressin; PTH = parathyroid hormone; numbers above the columns indicate quantities of each agent added per milliliter of cell suspensions; time of incubation was 20 min. Bracketed lines indicate SD.

experiments described above were found when kidneys from a different dog were used for the preparation of each series of renal-cell suspensions samples.

Further studies were carried out utilizing the renal-cell suspension system in order to test the specificity of the action of PTH and vasopressin on cyclic AMP production. When a group of duplicate samples was incubated with epinephrine and another with norepinephrine for 20 min, no significant rise in renal cyclic AMP was observed.

The possibility that PTH and vasopression have an additive effect on renal cyclic AMP was also examined. A group of duplicate samples was incubated with PTH, another with vasopressin, and a third with both PTH and vasopressin in the same concentration as the first two groups. There was an additive effect of PTH and vasopressin on renal cyclic AMP production in the samples in which both hormones were added.

Discussion. Several investigators have been utilizing intact kidney, slices, homogenates, and subcellular fractions to study the various physiological and biochemical functions of the kidney. In a recent report from our laboratory (3), we have described a renal-cell suspension system which is found suitable for such studies *in vitro*. In the present study, utilizing this new technique, we have found that the renal-cell suspension made of dog kidney cortex, showed an increased production of cyclic AMP with both PTH and pitressin. This cell system is devoid of hemodynamic and neurogenic influences.

It has been reported previously that PTH causes an increase in urinary excretion of cyclic AMP through a direct action of this hormone on the kidney (2), and it was suggested that cyclic AMP is the mediator of the action of this hormone. The studies reported here, utilizing a new approach, support the view that cyclic AMP is involved in the mechanism of action of PTH in the kidney and that this action is brought about through a direct effect on the metabolism of the renal cells. The studies reported here have also shown that PTH does not interfere with the cyclic AMP assay since addition of this hormone to the cell-suspension samples after the homogenization step of the procedure had no effect on the cyclic AMP concentration.

Incubation of cell suspensions with pitressin resulted in a rise in cyclic AMP concentration. A comparison between the cyclic AMP response to pitressin and PTH was also made. Although two doses were used and definitive conclusions can not be drawn, the rise of cyclic AMP concentration with pitressin was less than that obtained with PTH.

The observation that PTH and vasopressin have an additive effect on the renal cyclic AMP production suggests that these hormones act on different renal-cell populations.

The present studies show that the cell-suspension system (3) can be utilized for the *in vitro* studies of metabolic functions of intact renal cortical cells.

Summary. A cell-suspension system was utilized to study the effect of PTH and pitressin on the cyclic AMP concentration in renal cells. Cyclic AMP increased in the presence of either hormone. These findings support the view that cyclic AMP is involved in the mechanism of action of these hormones in the kidney and that this action might be brought about through a direct effect on the metabolism of the renal cells. It is suggested that the renal-cell suspension system might prove to be a versatile tool in renal physiology.

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