Urological Research 7, 281-283 (1979)

Urological Research

cAMP Content in Rat Urine Following 10 and 20 Days Oral Administration of a Calcium-Binding Ion Exchanger

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Accepted: February 2, 1979

Summary. Rats received a diet containing a Ca-binding ion exchanger at a dose of 30 and 90 g/kg diet, respectively. Following 10 days of oral administration there was a dose dependent increase in urinary cAMP excretion. However, after 20 days treatment the measured cAMP content in the urine was no longer different from control values. The results suggest that urinary cAMP excretion in the rat is only of value as an indication of acute changes in PTH-activity.

Key words: cAMP, Calcium metabolism, Parathyroid hormone, Ion-exchanger, Rat.

INTRODUCTION

The oral application of a Ca-binding ion exchanger has been increasingly recommended for the prophylaxis of recurrent Ca-urolithiasis (9). However, the resulting decrease in intestinal Ca-absorption may induce changes in the activities of the hormones involved in the regulation of the Ca concentration in body fluid.

One such hormone is Parathyroid Hormone (PTH), which is secreted from the parathyroid glands in response to a fall in the ionic Ca concentration of the blood. The direct determination of this hormone in serum raises several problems especially in rats, and therefore indirect methods are often used to determine its activity.

The amount of cAMP in the urine is considered to be a useful index of PTH activity (2, 4). The relationship between PTH activity and cAMP excretion is illustrated in Figure 1. cAMP is considered to be the intracellular mediator, or second messenger, of various hormones including PTH.

In renal cells PTH activates adenylate cyclase resulting in an increased formation of cAMP from ATP. The cAMP then activates other intracellular enzyme systems which ultimately trigger the hormonal effects. The cAMP formed is inactivated by phosphodiesterases. In PTH-sensitive renal cells however, elimination of cAMP through the cell membrane appears to be another important step in the inactivation process of the second messenger cAMP (3). Up to 60% of the total urinary cAMP is considered to result from the renal effects of PTH (1).

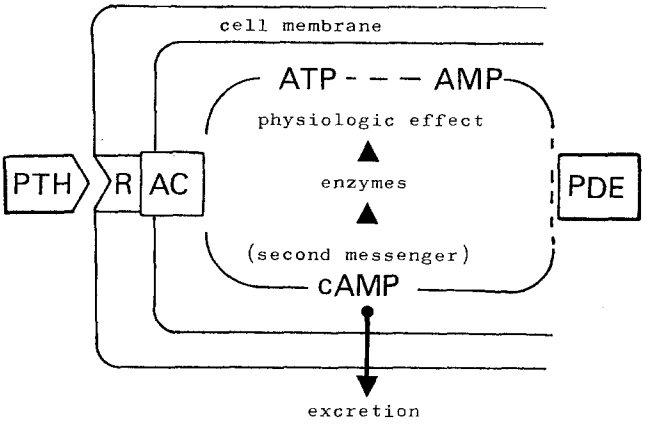

Fig. 1. Second messenger concept for renal effects of parathyroid hormone.
Abbreviations: PTH, parathyroid hormone; AC, adenylate cyclase; R, receptor; PDE, cyclic nucleotide phosphodiesterase

0300-5623/79/0007/0281/$01.00

Table 1. cAMP content (μmoles/g creatinine) in rat urine following long term oral treatment with a cation exchanger (CE). The values are the mean ± s.e.m. (Exp. animals N = 15, control animals N = 10)

g CE/kg diet	Control values before treatment	Treatment	
		10 days	20 days
0 (N = 10)	8.6 ± 0.8 (N = 40)	8.5 ± 1.2	7.7 ± 1.8
30 (N = 15)		17.9 ± 4.4*	13.3 ± 3.5
90 (N = 15)		24.0 ± 1.3**	11.0 ± 3.3

Statistical comparison with t-test: *2P<0.01, **2P<0.001.

Using cAMP excretion as an index of hormonal activity, we investigated whether the long term oral application of a Ca-binding ion exchanger influenced PTH activity.

MATERIAL AND METHODS

22 female Wistar rats, (starting weight 135 - 150 g) received a standard diet (Nafag Nr. 900, Nafag Gossau, SG, Switzerland) containing a cation exchanger (Campanyl, Temmler Werke, Marburg) at a dose of 30 and 90 g/kg diet, respectively. 21 control animals were pair fed with the experimental animals on the same diet without the addition of cation exchanger.

cAMP content was determined in the rat urine by a radioimmunoassay (10). Using an automatic pipetting station (Micromedic, model 24002) the following solutions were mixed in plastic tubes: 100 μl anti-cAMP rabbit serum (1:10, 000) containing normal rabbit serum (1:5, 000) as carrier, 200 μl goat anti-rabbit serum (1:80), 100 μl ^{125}J-O_2-monosuccinyl-cAMP-tyrosine methyl ester (10, 000 cpm) and 50 μl cAMP standards or rat urine diluted 1:200. For all solutions Na-acetate buffer 50 mM, pH = 6.4 was used. All determinations were performed in triplicate. After incubation for 17 hours at 2°C the tubes were centrifuged, the supernatant removed and the radioactivity of the sediment determined in a gamma-counter.

For the calculations a Hewlett-Packard computer (model 2100 S) was used (8). The creatinine content in urine was determined with an auto-analyser. (SMAC = High speed, computer-controlled biochemical analyser, Technicon Instruments Corporation, Tarrytown).

RESULTS

The results are summarised in Table 1. Before the application of the diet containing the cation exchanger, the average urinary cAMP excretion of all animals used in the experiment was 8.6 p moles/g creatinine. Following a daily oral application of the diet containing the cation exchanger in doses of 30 or 90 g/kg diet for 10 days there was a significant and dose dependent increase in the urinary cAMP excretion. When compared with control levels the increase was 109% (2 P < 0.01) and 180% (2 P < 0.001) in the group receiving the diet with 30 or 90 g cation exchanger/kg diet respectively. However, after a 20 day treatment with the cation exchanger the measured cAMP content was no longer statistically different from the control level.

DISCUSSION

The observed increase in urinary cAMP excretion following 10 days of treatment with an ion exchanger may be due to increased PTH activity. However, the normalisation after 20 days of oral treatment is difficult to understand. There are several possible explanations:

a) PTH-activity becomes normal as a consequence of adaptive changes in the intestinal Ca-absorption.
b) PTH-activity is increased, however the cAMP forming system has lost hormone-sensitivity resulting in smaller cAMP formation.
c) Intracellular degradation of cAMP is increased as a result of increased phosphodiesterase activity.
d) The membrane permeability for cAMP has decreased.

In the same experiment other parameters for PTH activity such as Ca-turnover or phosphate excretion in urine have been determined following a 20 day treatment with the ion exchanger. These results are reported separately (6).

It was found that Ca-turnover was increased and urinary phosphate excretion decreased. Such changes are considered to result from increased PTH-activity. Therefore it seems that the observed normalisation of the urinary cAMP excretion after 20 days of treatment with an ion exchanger may result from changes in the cAMP system, and not from a normalisation of PTH-activity. Of the remaining possibilities such as increased intracellular degradation, decreased membrane permeability or loss of hormone sensitivity the latter seems to be the most probable. In several receptor controlled systems a loss of hormone sensitivity has been observed after prolonged stimulation (5, 10). The other possibilities cannot, however, be excluded. Further experiments are necessary to clarify this point.

In conclusion, the experiments show that oral administration of an ion exchanger leads to an increased urinary cAMP excretion in rats, which

may result from an increased PTH activity. However, the observed normalisation of cAMP excretion after longer oral administration suggests that the amount of cAMP excreted in urine may only be of value as an indication of acute changes in PTH-activity.

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