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Effect of parathyroid hormone and adenosine 3′,5′-monophosphate on renal carbonic anhydrase*

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Parathyroid hormone (PTH) increases the renal excretion of phosphate [1]. In addition, PTH causes a modest increase (up to about 7 per cent of filtered load) of bicarbonate excretion [2]. It has been postulated that the effect of PTH and cyclic AMP, which also mimics the action of PTH on phosphate excretion, produces excretion of bicarbonate by direct inhibition of renal carbonic anhydrase [3]. PTH (10 units/ml) and cyclic AMP (0·4 mM) have been reported to inhibit rat renal carbonic anhydrase, after 20 min pre-incubation of the homogenate at 37°, up to 33 and 60 per cent respectively [3]. It has been further reported that 0·4 mM cyclic AMP but not PTH produced 85 per cent inhibition of carbonic anhydrase purified from rat renal cortex after 2-min pre-incubation [4]. However, Puschett and Goldberg [2] reported lack of effect of PTH on erythrocyte carbonic anhydrase. Beck and Goldberg [5] did not find any inhibition of erythrocyte carbonic anhydrase with 150 units/ml of PTH or 3 mM cyclic AMP. Therefore, it was thought to be of interest to reinvestigate the effect of PTH and cyclic AMP on renal carbonic anhydrase.

Table 1. Effect of parathyroid hormone on carbonic anhydrase in buffered supernatant fraction of rat renal cortex*

Parathyroid hormone (units/ml)	Carbonic anhydrase† (c.u./ml)	pH
0	1·89 ± 0·10	7·32
10	1·85 ± 0·12	5·92

* Samples were incubated for 20 min at 37° and the pH was recorded after cooling the samples.

† Values given here are mean ± standard deviation of three experiments.

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Rats of the Wistar strain were anesthetized with ether, and their kidneys were perfused free of blood with 0·9% NaCl solution through renal arteries. The medulla and cortex were separated, weighed and homogenized in 10 vol. of 50 mM Tris buffer, pH 7·4, or distilled water with a glass homogenizer. The homogenates were centrifuged at 3000 rev/min for 30 min in a Sorvall (model RC-2B) centrifuge.

The supernatant fraction was mixed with PTH or acetic acid or a freshly prepared solution of cyclic AMP. The samples were incubated at 37° in a water bath with a shaker for 20 min. After incubation, the samples were cooled and their pH was recorded. The carbonic anhydrase activity of the samples was then determined by the method of Maren *et al.* [6]. The variability of the method was 10 per cent.

PTH used was Parathyroid Injection of Eli Lilly & Co. Cyclic AMP was obtained from Sigma Chemical Co.

Table 1 shows that incubation of renal cortical carbonic anhydrase with 10 units/ml of parathyroid hormone had no effect on the enzyme activity, when the enzyme was in a buffer and the pH did not drop below 5. It may be pointed out that the pH of the Parathyroid Injection (containing 100 units/ml) was 3·3. In the case of enzyme prepared in distilled water, its pH dropped to 4·6 after adding 10 units/ml of parathyroid hormone (Table 2). In this case, the incubation of the reaction mixture for 20 min at 37° produced a 33 per cent decrease in carbonic anhydrase activity. The sample without pre-incubation did not show any change in enzyme activity. The results of Table 2 suggest that acidic

Table 2. Effect of parathyroid hormone on carbonic anhydrase in non-buffered supernatant fractions of rat renal cortex

Parathyroid hormone (units/ml)	Carbonic anhydrase* (e.u./ml)	% Change from control	pH
0	1·25 ± 0·05†		7·25
10	0·83 ± 0·02†	−33	4·60
0	1·25 ± 0·06‡		7·25
10	1·35 ± 0·07‡	+ 8	4·60

* Carbonic anhydrase values are mean ± standard deviation of three experiments.
† After 20-min pre-incubation at 37°.
‡ No pre-incubation.

Table 3. Effect of acid on renal carbonic anhydrase

pH	Carbonic anhydrase* (e.u./ml)	% Change from control
7·0	2·40 ± 0·2†	
4·6	1·50 ± 0·3†	37
4·6	2·41 ± 0·22‡	0

* Carbonic anhydrase values are mean ± standard deviation of three experiments.
† After 20-min pre-incubation at 37°.
‡ No pre-incubation.

pH inactivates the enzyme during incubation. This is confirmed from the data given in Table 3 where the pH of the two samples containing renal carbonic anhydrase was lowered by acetic acid to 4·6. In this case, the incubated sample showed a 37 per cent decrease in enzyme activity.

Table 4 shows that the neutral solution of cyclic AMP did not produce any effect on renal carbonic anhydrase. In this case, the pH of the samples was between 7·1 and 7·4. However, when the pH of the cyclic AMP solution (3·0) was not adjusted and the enzyme was prepared in distilled water, 0·66 mM cyclic AMP produced a 44 per cent decrease in carbonic anhydrase activity. The pH was 4·4 (Table 5). When the pH of cyclic AMP solution was raised to 4·3 before addition to renal carbonic anhydrase, 6·66 mM but not 3·33 mM cyclic AMP produced a significant change in enzyme activity.

The results of the present investigation are in agreement with Goldberg *et al.* [2, 5] but not with those of Beck *et al.* [3, 4]. The pH of the parathyroid hormone and cyclic AMP solutions was not given nor was the effect known of these substances on the pH of the carbonic anhydrase samples. The present results clearly show that, if the pH of the enzyme solutions kept above 5 to avoid inactivation of the enzyme, parathyroid hormone or cyclic AMP has no effect on renal carbonic anhydrase of rat. Byvoet and Gotti [7] have shown that the electrophoretic mobility and inhibition kinetics of carbonic anhydrase from dog kidney are similar to that of dog erythrocyte enzyme. In fact, there is no known example of any tissue specificity with respect to inhibition of carbonic anhydrase with the single exception of liver carbonic anhydrase in certain rodents [8–10] which is relatively resistant to sulfonamides and is affected by sex hormones.

The actions of PTH and carbonic anhydrase inhibition are superficially similar but are both qualitatively and quantitatively different. PTH barely alkalinizes the urine, while the effect of acetazolamide on urinary phosphate is to double it, at the most [2, 11, 12].

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Table 4. Effect of neutral solutions of cyclic AMP on renal carbonic anhydrase

Cyclic AMP (mM)	Carbonic anhydrase* (e.u./ml) Renal cortex	Renal medulla
0	2·04 ± 0·08	3·38 ± 0·05
2	1·91 ± 0·08	3·42 ± 0·07
4	1·89 ± 0·10	
6	1·88 ± 0·11	3·42 ± 0·08

* Values given are mean ± standard deviation of three experiments.

Table 5. Effect of acidic solutions of cyclic AMP on carbonic anhydrase from renal cortex

Cyclic AMP (mM)	Carbonic anhydrase (e.u./ml)	% Change from control	pH
0	0·81 ± 0·02		6·5
0·33*	0·87 ± 0·03	+ 7·4	5·3
0·66*	0·45 ± 0·01	−44·4	4·4
0	1·68 ± 0·04		5·9
3·33†	1·63 ± 0·04	− 3·0	4·8
6·66†	0·80 ± 0·02	−52·0	4·6

* The pH of cyclic AMP solution used was 3·0.
† The pH of the cyclic AMP solution was raised to 4·3.

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Incorporation of labelled amino acids into liver protein after acute ethanol administration

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Chronic consumption of ethanol was accompanied by reduced incorporation of labelled amino acids into rat liver protein measured *in vivo* [1–3] and in a perfused system [3, 4]. The present experiments were performed to study the effect of acute administration of ethanol on the incorporation of labelled amino acids into liver protein. Previous results on the effect of acute ethanol treatment on the synthesis of fixed liver proteins are controversial [5–8]. This could have been due to effects of ethanol on the uptake of amino acids [5, 9, 10], or on the hepatic metabolism of amino acids [11, 12] which could have changed the specific radioactivity of the labelled precursor used in those incorporation experiments. Therefore, in the present study the incorporation was studied in experiments which allowed us to assess if ethanol influenced the precursor pools.

Male albino Wistar rats (240–350 g final body wt) were fed either ordinary laboratory chow plus water or in long-term experiments a special diet [3, 4] providing approximately 11 g ethanol per kg rat and day for 4 weeks. In this diet, ethanol calories constituted about 25 per cent of the total calories consumed. In long-term control diets, sucrose replaced ethanol isocalorically. All animals were fasted 24 hr before sacrifice. Liver perfusions were conducted as described elsewhere [13, 14]. The pH of recirculating perfusates (40 ml) was maintained at 7·40 ± 0·02 by additions of 0·5 N $NaHCO_3$ from an autoburette monitored by a titrator (ABU-13, Titrator 11, Radiometer, Copenhagen) coupled to a pH-electrode (pH-meter 28, Radiometer, Copenhagen) reading perfusate pH continually. Ethanol (19·2%, v/v) was added after perfusion for 5 min followed by infusion of a 12·8 per cent solution to maintain the desired ethanol concentration, infusion rate varying from 15 to 30 μl/min. Equal amounts of perfusion buffer were added instead of ethanol to control perfusates. More 0·5 N $NaHCO_3$ was added to ethanol perfusates than to control perfusates to keep pH at 7·40 [15]. 0·5 M NaCl was therefore added to the latter perfusates to obtain an equal dilution. 50 μCi of [2,3-^{3}H] L-valine (TRK. 327, 39 Ci/m-mole, The Radiochemical Centre, Amersham) was added to the perfusates after 15 min and cycloheximide (Sigma) was added after perfusion for 1 hr, final concentration, 20 μg/ml. Plasma from 1 ml of perfusate was separated at times indicated and frozen at −20°. A liver lobe was ligated and removed after 1 hr, and several liver samples were taken at the end of perfusion. All samples were frozen in liquid nitrogen and stored at −20° until analysis. Ethanol (0·25 g/ml 0·15 M NaCl) 3·3 g/kg was injected intraperitoneally into some rats 4 hr before sacrifice, while controls were given an injection of saline only. All these rats received 5 μCi/kg of [U-^{14}C] L-leucine (CFB. 67, 311 mCi/m-mole, The Radiochemical Centre, Amersham) by the same route 3 hr later, 1 hr before decapitation, when liver samples were taken, frozen and stored as described above. Blood was collected from the neck vessels. Ethanol concentrations were determined by gas chromatography in perfusate (every 30 min) and in blood at sacrifice. The concentration of free unlabelled leucine in liver was measured* after precipitation of liver proteins with 3 vol ice-cold 10% (w/w) trichloroacetic acid (TCA). The supernatants were diluted with 9 vol 0·2 M sodium citrate pH 2·2 containing 0·5% (v/v) thiodiglycol and 0·01% (v/v) caprylic

* This determination was kindly performed by Dr. Hans Prydz, University of Tromsø.