THE EFFECT OF IN VIVO TREATMENT WITH EHDP AND/OR 1,25-DHCC ON CALCIUM UPTAKE AND RELEASE IN ISOLATED KIDNEY MITOCHONDRIA [+]

D.F. Guilland and H. Fleisch *

Department of Pathophysiology, University, Berne, Switzerland

Received October 25,1974

Previous work on calcium transport (uptake and release) in isolated mitochondria, in vitro, has shown that addition of EHDP to the medium does not influence calcium uptake, but does delay calcium release. In vivo treatment of normal chicks with high doses of EHDP (10 mg P/kg body weight/day) has now also been found not to affect the in vitro calcium uptake in isolated chick kidney mitochondria, but to delay the subsequent release as compared with controls. The effect is not due to the decrease in 1,25-DHCC, since chronic administration of this metabolite did not correct the delay. In fact 1,25-DHCC in itself had a delaying effect on accumulated calcium release.

INTRODUCTION

Diphosphonates, compounds possessing a P-C-P grouping, have been shown to exert profound effects on calcium metabolism. Thus these compounds inhibit experimental soft tissue calcification (1) and diminish bone resorption (2). These effects have until recently been thought to be explained mainly through physico-chemical mechanisms (3). Some evidence, however, suggests that these compounds may also have a biological effect.

[+] This work has been supported by the Schweizerischer Nationalfonds für wissenschaftliche Forschung (3.326.70), by the U.S. National Institutes of Health (AM 07266), and by the Procter and Gamble Company, USA.

* To whom to address correspondence.

Thus addition of ethane-1-hydroxy-1,1-diphosphonate (EHDP), in vitro, to mitochondrial suspensions from normal animals, delays release of accumulated calcium (4). We have now studied whether EHDP given at high doses (10 mg P/kg b.w.) to animals in vivo has a similar effect on the in vitro calcium transport in isolated mitochondria. Since such doses of EHDP are known to depress the in vivo synthesis of the D-metabolite 1,25-dihydroxycholecalciferol (1,25-DHCC) (5,6), we have studied whether the effect of EHDP can be counteracted by the administration of the active metabolite, 1,25-DHCC, in this system.

MATERIALS AND METHODS

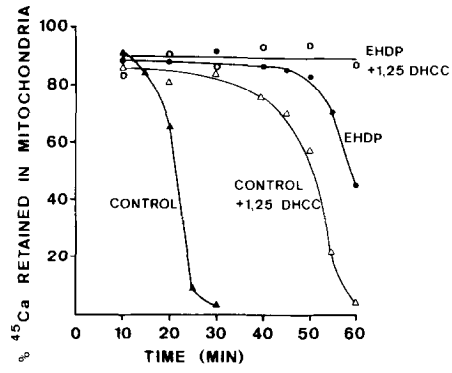
One day old male white leghorn chicks were raised on a stock commercial diet (Seg 21, obtained from V.L.G. Berne, Switzerland) and treatment was started on the 15th day. The animals were divided into four groups: control, receiving daily injections of 0.15 M NaCl (0.1 ml/100 g b.w.) s.c. and 50 µl 96 % ethanol i.v. or i.p.; control-1,25-$(OH)_2D_3$, receiving a similar amount of NaCl and also 13 pmoles 1,25-$(OH)_2D_3$ in 50 µl ethanol; EHDP, receiving 10 mg P/kg/day in saline (0.1 ml/100 g b.w.) and 50 µl 96 % ethanol; EHDP-1,25-$(OH)_2D_3$, receiving a similar dose of EHDP and 13 pmoles of 1,25-$(OH)_2D_3$ daily. Injections were continued for 1 week and the animals were sacrificed by decapitation 24 hours after the last injection of EHDP/saline. The blood was collected in heparinised tubes and calcium was determined by atomic absorption and phosphate colorimetrically as described by Chen (7). The kidneys were quickly removed, washed in ice-cold 0.25 M sucrose (pH 7.4) and carefully dissected for re-

moval of extraneous material. The mitochondria were prepared according to the method of DeLuca and Engstrom (8) and the incubation medium and conditions were as previously described (4). The ATP concentration was 0.06 mM in all experiments. The method of sampling, filtration through Millipore and calculation of the % ^{45}Ca retained in the mitochondria at any one time has also been described (4). In two experiments 5 pmoles of 1,25-$(OH)_2D_3$ in 20 µl of 96 % ethanol were added to the medium to a control and an EHDP preparation 20 minutes after the initation of the experiment, to test the in vitro effect of the D-metabolite.

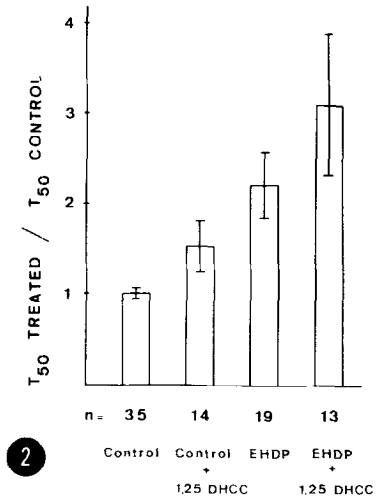
RESULTS

No difference in plasma calcium could be detected in any of the four groups, however, EHDP-treated animals were significantly hypophosphatemic (control 7.5 $\pm$ 0.3, EHDP 5.9 $\pm$ 0.3, $p < 0.001$), a factor which was partially corrected by the administration of 1,25-$(OH)_2D_3$ (EHDP-1,25-$(OH)_2D_3$ 6.2 $\pm$ 0.4).

No effect of treatment on the total amount of calcium accumulated in the mitochondria at the end of 5 minutes, could be detected. The release on the other hand was significantly changed. The pattern obtained from one typical experiment is shown in Fig. 1. In vivo treatment with EHDP delays release of accumulated mitochondrial calcium in vitro. The administration of 1,25-DHCC also delays the release whether given alone or in combination with EHDP. This figure also serves to explain the derivation of the term T_{50} which is the time in minutes when 50 % of the calcium has been released to the medium. Fig. 2 shows all the results obtained. The T_{50} values have been expressed as the ratio con-



1



2

Fig. 1

Effect of in vivo treatment with 10 mg P/kg b.w. EHDP and/or 1,25-DHCC 13 pmoles/day on the release of accumulated mitochondrial calcium in vitro. The results are those of one typical experiment and also serve to show how T_{50} values were obtained.

Fig. 2

Effect of EHDP and/or 1,25-DHCC on the time in minutes where 50 % of the accumulated calcium has been released to the medium (T_{50}). Each separate mitochondrial suspension (1 chick) was studied in triplicate and the T_{50} value was calculated from the average of the three T_{50} obtained by plotting the three individual time curves. In experiments where no release was observed, the T_{50} is taken as the time when the last sample of the experiment was taken. The results are expressed as T_{50} treated / T_{50} control and are the combined results of all experiments, $\pm$ 2 SE. n = number of chicks.

trol: treated because of the variation in control T_{50}. It appears again that both EHDP and 1,25-DHCC delay calcium release.

DISCUSSION

The delay in release of accumulated calcium from chick kidney mitochondria treated in vivo with high amounts of the diphosphonate EHDP, agrees with previously found effects of EHDP in vitro

(4). This action of EHDP, therefore, supports the hypothesis that some effects of diphosphonates may be due to cellular rather than to pure physico-chemical effects.

In vivo treatment with high doses of EHDP has been shown to lower endogenous production of 1,25-DHCC in the rat and chick (5,6). One physiological effect of high EHDP, the decreased intestinal absorption of calcium, can be corrected by a daily dose of 13 pmoles 1,25-DHCC (9). It was therefore thought possible that the delay in calcium release from mitochondria in vitro induced by EHDP, could also be corrected by daily administration of 1,25-DHCC. This however was not found to be the case. Not only did 1,25-DHCC alone result in delayed calcium release as compared with control, but a combination of EHDP and 1,25-DHCC resulted in an even greater delay. The mechanism of this effect of 1,25-DHCC is unknown. It is tempting to speculate that some of the physiological effects of 1,25-DHCC involve this mitochondrial effect.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. H.F. DeLuca for giving us the 1,25-DHCC and Miss A. Monod and Mr. D. Mousley for their valuable technical assistance.

1. Fleisch, H., Russell, R.G.G., Bisaz, S., Mühlbauer, R.C. and Williams, D.A. (1970) Europ.J.Clin.Invest. 1, 12-18.
2. Russell, R.G.G., Mühlbauer, R.C., Bisaz, S., Williams, D.A. and Fleisch, H. (1970) Calc.Tiss.Res. 6, 183-196.
3. Francis, M.D. (1969) Calc.Tiss.Res. 3, 151-162.

4. Guilland, D.F., Sallis, J.D. and Fleisch, H. (1974) Calc. Tiss.Res. in press.

5. Hill, L.F., Lumb, G.A., Mawer, E.B. and Stanbury, S.W. (1973) Clin.Sci. 44, 335-347.

6. Baxter, L.A., DeLuca, H.F., Bonjour, J.-P. and Fleisch, H. (1974) Arch.Biochem.Biophys. in press.

7. Chen, P.S., Toribara, T.Y. and Warner, H. (1956) Anal. Chem. 28, 1756-1758.

8. DeLuca, H.F. and Engstrom, G.W. (1961) Proc.natl.Acad.Sci. 47, 1744-1750.

9. Bonjour, J.-P., DeLuca, H.F., Fleisch, H., Trechsel, U., Matejowec, L.A. and Omdahl, J.L. (1973) Europ.J.Clin.Invest. 3, 44-48.