Effects of 1.25 dihydroxyvitamin D_3 on calcium uptake, into rat intestine after chronic oral cadmium administration[1]

M. J. Fauran-Clavel, J. Oustrin and F. Fauran

Laboratoire de Physique, Faculté des Sciences Pharmaceutiques, 31, allées Jules Guesde, F-31077 Toulouse cedex (France), 15 October 1979

Summary. Calcium influx into rat intestine was investigated, in vitro, after cadmium chronic oral exposure. In active calcium transport, pretreatment by 1.25 dihydroxyvitamin D_3 prevents the effect of cadmium on the maximal influx value J_m and leads to an increase of the half-saturation constant Kt.

In previous work[2] the authors demonstrated a significant decrease of calcium absorption in the rat intestine following 6 weeks' oral cadmium administration. An in vitro investigation of the transport mechanism of calcium[3] showed that only the saturable term of calcium influx (active transport) was affected and could be related to the reduction of total calcium influx. In this study we examine, in animals chronically exposed to cadmium, effects on calcium influx of an oral 1.25 dihydroxyvitamin D_3 treatment. In vivo this metabolite of vitamin D_3, which results from 1.25 hydroxylation in the liver and then an 1-α-hydroxylation in kidney mitochondria, acts directly on intestinal calcium transport[4-7].

Materials and methods. Experiments were carried out on 4 groups of 24 female Wistar rats approximately 2 months old at the start of treatment, and on a normal diet, as follows: group I (cadmium-exposed). These animals were administered 8 mg of cadmium in form of dihydrate acetate/kg b.wt, 6 days out of 7, and this by oesophageal intubation. Group II (cadmium-exposed + 1.25 $(OH)_2D_3$). These rats were treated in the same way as those of group I, but in addition received 80 ng/kg/day of 1.25 dihydroxyvitamin D_3 in a solution of olive oil. This was administered 3 h after the cadmium and only 5 days out of 7. Group III (1.25 $(OH)_2D_3$). This group was administered only 80 ng/kg/day of 1.25 dihydroxyvitamin D_3, 5 days out of 7. Group IV was control group.

The cadmium and 1.25 $(OH)_2D_3$ were administered at the same time for the different groups. The animals were not put on a special diet before the experiment, and can be considered to be in identical condition as regards absorption. For the first 3 groups, the calcium influx was measured 24 h after administration of the last dose of 1.25 $(OH)_2D_3$.

The kinetics of calcium influx were measured according to Papworth's method[8] which was adapted for our experiment[3]. 12 duodenal slices were prepared from each rat, 1,5-5 cm from the pylorus. In each flask 6 slices (total fresh wt approximately 200 mg) were preincubated for 15 min in 8.1 ml Ca-free medium containing 139.4 mM NaCl, 4.7 mM Tris buffer at pH 7.4 and 27.8 mM glucose. Then 10.5 ml medium was added with some Na replaced isomotically by Ca, labelled with ^{45}Ca (as $CaCl_2$ with sp. act. 19.5 mCi/mg). After 5 min further incubation, the slices were washed twice with 154 mM NaCl, blotted and weighed. The uptake of ^{45}Ca was measured by liquid scintillation. Each value gave influx per flask of 6 slices of intestine, with 1 originating from each site but taken at random from 6 rats.

Results. According to Papworth et al.[8], Ca influx over a range of calcium concentrations was analyzed into saturable (J_1) and linear (J_2) components as follows:

$$J = \frac{Jm[Ca]}{Kt + [Ca]} + P[Ca] \qquad (1)$$

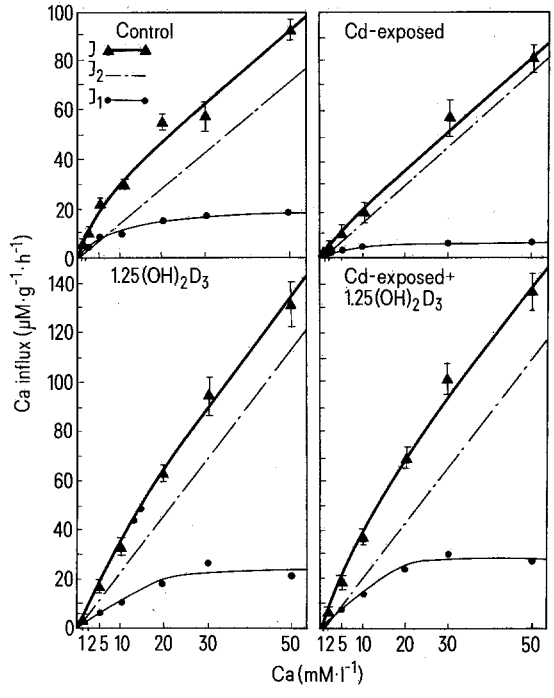

Fig. 1. Ca influx into duodenal slices under standard conditions in vitro. J, total Ca influx; J_1, saturable component (active transport); J_2, linear component (passive transport). Means ± SE of 4 experiments.

Table 1. Total calcium influx beyond maximal value of saturable component: Linear regression equations, constant of passive transport P and maximal calcium influx value Jm (active transport)

	Regression equation ($J^* = P[Ca] + Jm$)	$P(ml \cdot g^{-1} \cdot h^{-1})$**	$Jm(\mu M \cdot g^{-1} \cdot h^{-1})$**
Control	$J = 1.42[Ca] + 22$	1.42 ± 0.22	22 ± 8
Cd-exposed	$J = 1.56[Ca] + 6$	1.56 ± 0.21	6 ± 6
Cd-exposed + 1.25$(OH)_2D_3$	$J = 2.21[Ca] + 28$	2.21 ± 0.29	28 ± 10
1.25$(OH)_2D_3$	$J = 2.24[Ca] + 22$	2.24 ± 0.33	22 ± 13

* Relationship and linear regression between variables, tested by Student's t-test and variance analysis, are accepted with a probability of error $\alpha \ll 0.05$. ** ± SE. Regression line equations are calculated using the method of least squares.

J = Ca influx, Ca = Ca concentration, Jm = maximum value of Ca influx, Kt = half-saturation constant for saturable component (carrier), P = constant for linear (simple diffusion) component, and:

$$J_1 = \frac{Jm[Ca]}{Kt+[Ca]}, \qquad J_2 = P[Ca]$$

from the resulting curves (figures 1 and 2), the following may be concluded: a) Both transfer mechanisms are involved in the experiments of these figures in which doses used ranged from 1 to 50 mM. b) The saturable component reaches its maximum level at 20 mM concentration for group II (cadmium-exposed + 1.25 $(OH)_2D_3$), group III (1.25 $(OH)_2D_3$) and group IV (control group) and at 10 mM for group I (cadmium-exposed). The curve becomes linear above these values. The expression (1) may be set down as:

$$J = Jm + P[Ca] = Jm + J_2 . \qquad (2)$$

By calculating the regression lines on the corresponding experimental points, the values of P and Jm may be determined (table 1). Comparisons were subsequently made for P (slope of regression line) and Jm (ordinate at its origin) in table 2. The only significant difference to appear as regards Jm were between the control group/Cd-exposed group, and the Cd-exposed/Cd-exposed + 1.25 $(OH)_2D_3$ groups.

J_1 was then determined for a given calcium concentration by the difference between total calcium influx J and the influx due to the linear phenomenon J_2, as follows: $J_1 = J - J_2 = J - P[Ca]$.

The Linewaver-Burk plot was used to study the 2 parameters Kt and Jm (figure 2). The lines obtained for groups II and III (animals treated with 1.25 $(OH_2D_3$ passed almost through the origin, so it was not possible to give the values of Jm and Kt from their reciprocals with any degree of accuracy (table 3). However, it was possible to compare the slopes and ordinates at the origin of the 4 regressions in double reciprocal plots. This showed non-significant difference for the ordinates at origin (1/Jm) between the control group, the Cd-exposed + 1.25 $(OH)_2D_3$ group and the 1.25 $(OH)_2D_3$ group, but significant differences in the slopes between control/treated 1.25 $(OH)_2D_3$ and Cd-exposed/Cd-exposed + 1.25 $(OH)_2D_3$ groups. The results imply a significant difference between the abscissa at origin 1/Kt, therefore between the Kt. For Jm the comparison

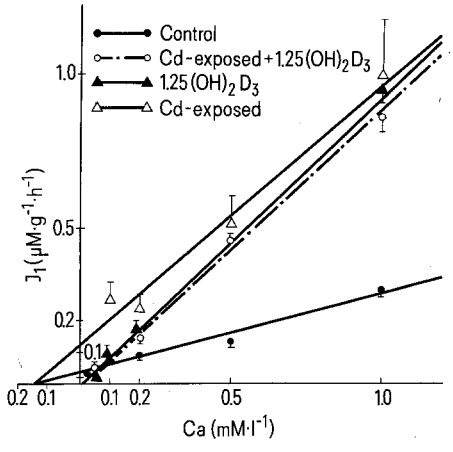

Fig. 2. Lineweaver Burk plots are fitted by linear regression; Means ± SE of 4 inverses values are reported.

Table 2. Results of comparison of regressions

Comparison	p*	Jm**	Kt***
Control / Cd-exposed	N.S.	S. $Jm_1 > Jm_2$	N.S.
Control / Cd-exposed + 1.25 $(OH)_2D_3$	N.S.	N.S.	S. $Kt_1 < Kt_2$
Control / 1.25 $(OH)_2D_3$	N.S.	N.S.	S. $Kt_1 < Kt_2$
Cd-exposed / Cd-exposed + 1.25 $(OH)_2D_3$	N.S.	S. $Jm_1 < Jm_2$	S. $Kt_1 < Kt_2$
Cd-exposed + 1.25 $(OH)_2D_3$ / 1.25 $(OH)_2D_3$	N.S.	N.S.	N.S.

* P comparison issue from 1 analysis regression set of data (table 1). ** Jm comparisons issue from 2 analysis regression sets of data (tables 1 and 3) - Indexes (1) and (2) are respectively related to the 1 and 2 term of the comparison. *** Kt comparison issue from 2 analysis regression set of data (table 2) - Indexes (1) and (2) are respectively related to the 1st and 2nd term of comparison. N.S., Non-significant difference at the 5% level. S., Significant difference at the 5% level.

Table 3. Active transport (J_1): Linear regression equations, maximal calcium influx value (Jm) and half. Saturation constant (Kt) according to the method of Lineweaver and Burk

	Regression equations $\frac{1^*}{J1} = \frac{Kt}{Jm} \cdot \frac{1}{[Ca]} + \frac{1}{Jm}$	Jm ($\mu M \cdot g^{-1} \cdot h^{-1}$)	Kt ($mM \cdot h^{-1}$)
Control	$\frac{1}{J_1} = 0.25 \cdot \frac{1}{[Ca]} + 0.0304$	33 (17-294)**	8 (6-11)**
Cd-exposed	$\frac{1}{J_1} = 0.86 \cdot \frac{1}{[Ca]} + 0.127$	8 (0-23)**	6,8 (0-20)**
Cd-exposed + 1.25 $(OH)_2D_3$	$\frac{1}{J_1} = 0.89 \cdot \frac{1}{[Ca]} + 0.023$	***	***
1.25 $(OH)_2D_3$	$\frac{1}{J_1} = 0.94 \cdot \frac{1}{[Ca]} - 0.011$	***	***

* Relationship and linear regression between variables are accepted with a probability of error $\alpha \leq 0.05$. ** 95% confidence intervals. *** 1/Jm and 1/Kt values are very close at the origin, Jm and Kt tend towards indeterminately high values. Regression line equations are calculated using the method of least squares.

thus confirms the results already gained from the equation (2).

Discussion. The method used seems to be sufficient for our purpose, though O'Donnell[9] took exception to it because it does not limit the contact of the calcium solution to the mucosa alone. However, kinetic studies[8] in the normal rat led to the conclusion that the saturable component for slice influx could be ascribed mainly to transport across the mucosal surface. The similar estimate of linear component P of Ca influx across only the mucosal or the serosal surfaces reflects a simple diffusion into tissues. In this study, analysis of this linear component does not show change of P in any case. In both the Cd-exposed and the 1.25 $(OH)_2D_3$-treated control group, the apparent increase of J_2 is not confirmed by statistical analysis. This fact may be due to the complexity of the system under study and experimental values dispersion. Administration of 1.25 dihydroxyvitamin D_3 to exposed animals restores Jm to a value comparable to that in control and 1.25 $(OH)_2D_3$ groups. Furthermore the experiments show a peculiar effect of 1.25 dihydroxyvitamin D_3 on the half-saturation constant Kt which is increased in all groups 1.25 $(OH)_2D_3$ dosed. These results express the double impact of this vitamin D_3 metabolite[10] on the turn-over activity of the carrier decreased by Cd^{++} intoxication, and on the affinity of the carrier in all cases. Little is known about the mechanism of an action of 1.25 dihydroxyvitamin D_3 on the intestinal absorption. To De Luca[11] its appears to be similar to the action of classic steroid hormones on their target tissue. Active calcium transport decrease in cadmium-intoxicated rats may result from a direct calcium-cadmium interaction[12] on the intestinal calcium carrier, or from an inactivation of 25 $(OH)D_3$ 1-α-hydroxylase in the kidney[13].

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Inactivation of yeast glucose-6-P dehydrogenase by aspirin[1]

P.F. Han, G.Y. Han, H.C. McBay and J. Johnson, Jr

Department of Chemistry, Atlanta University, Atlanta (Georgia 30314, USA), and Department of Chemistry, Morehouse College, Atlanta (Georgia 30314, USA), 31 January 1980

Summary. Glucose-6-P dehydrogenase is irreversibly inactivated by treatment with Na salts of aspirin. Kinetic data show that 1 molecule of aspirin reacts with each active unit when the enzyme is inactivated. The rate of inactivation is enhanced with increasing pH but is reduced in the presence of glucose-6-P or $NADP^+$. Na salicylate fails to inactivate the enzyme.

Although aspirin (acetylsalicylic acid) has been known to acetylate numerous biological macromolecules for more than a decade[2], studies on the functional consequences of treating enzymes with aspirin have largely been neglected with the exception of recent interest in prostaglandin synthetase[3]. We recently showed that the sensitivity of a fructose-1,6-bisphosphatase to allosteric inhibition by adenosine-5'-monophosphate was irreversibly modified after treatment with aspirin[4]. In this communication we report the irreversible inactivation of a glucose-6-P dehydrogenase (EC 1.1.1.49) by aspirin.

Materials and methods. Aspirin, crystalline glucose-6-P dehydrogenase from baker's yeast (270 units/mg of protein), and other chemicals used in this study were all purchased from Sigma. The activity of glucose-6-P dehydrogenase was assayed at 25 °C by measuring the rate of $NADP^+$ reduction at 340 nm. The standard reaction mixture (1 ml) contained 50 mM Tris-HCl buffer (pH 7.5), 0.25 mM $NADP^+$, 2 mM $MgCl_2$, 0.6 mM glucose-6-P, and an appropriate amount of glucose-6-P dehydrogenase. Reactions were initiated by addition of glucose-6-P dehydrogenase. Treatment of the enzyme (12 µg/ml) with different concentrations of Na salt of aspirin was carried out at 25 °C in 70 mM Tris-HCl buffer at the pH-values indicated in the figure and the table legends. Aliquots were removed at the times specified and immediately assayed for enzyme activity.

Results and discussion. Treatment of yeast glucose-6-P dehydrogenase with Na salt of aspirin resulted in rapid inactivation of the enzyme. The results obtained with various concentrations of aspirin are presented in figure 1,A, as semilogarithmic plot. The inactivation reaction follow pseudo-first order kinetics until about 90% of the enzyme had been inactivated, and prolonged incubation led to complete inactivation. The inactivation reaction

Effect of pH on the inactivation of yeast glucose-6-P dehydrogenase by aspirin*

pH	Half-time for inactivation ($t_{0.5}$) (min)
8.5	2.3
8.0	4.5
7.5	8.9
7.0	29.3
6.5	62.4
6.0	184.3

*Enzyme (12 µg/ml) was incubated at 25 °C with 10 mM Na salt of aspirin in 70 mM Tris-HCl buffer at the pH-values indicated. Portions were removed at specific times and assayed for enzyme activities as described in materials and methods. Times for 50% inactivation ($t_{0.5}$) were determined from semilogarithmic graphs in which residual activity was plotted against time as in figure 1,A.