Putrescine may be a natural stimulator of glucose-6-phosphate dehydrogenase

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The possible relationship between pyridoxal phosphate-dependent ornithine decarboxylase (ODC) activity and glucose-6-phosphate dehydrogenase (G6PD) activity has been studied in the osteoblasts of the growth-plate of metatarsals of rats fed a pyridoxine-deficient diet, which caused depressed G6PD levels. The G6PD activity was fully restored when it was assayed in the presence of putrescine. It is suggested that this relationship may account for the correlation generally found between growth and ODC activity.

Putrescine *Glucose-6-phosphate dehydrogenase* *(Osteoblast)* *Ornithine decarboxylase* *Growth*

1. INTRODUCTION

Proliferation and growth [1], which involve pentose-shunt activity [2], and also the effect of many trophic polypeptide hormones [3], are associated with stimulation of pyridoxal phosphate-dependent ornithine decarboxylase (ODC) activity. However the precise role of polyamines, generated from ODC activity, remains obscure. To examine the potential role of the immediate product of ODC activity, namely putrescine, we have concentrated on the activity of glucose-6-phosphate dehydrogenase (G6PD) which is the regulatory enzyme of the oxidative part of the pentose-phosphate shunt [4] and which is amenable to 'fine-tuning' responses [5] both by changes in its multimeric form [6] and by other regulatory mechanisms [7–9]. For this purpose we used quantitative cytochemical procedures on the growth-plate of the rat metatarsal for the following reasons: (i) we had previously shown that the G6PD activity of the growing osteoblasts was very susceptible to modulation [10,11]; (ii) because we were aware that the extraction of this enzyme from intact tissue could markedly alter its physicochemical characteristics [12]; (iii) because two groups had shown independently [13,14] that the polyamine content of various tissues of young Wistar rats can be depleted (to 50% of normal values in the liver), when fed for 21–60 days on a particular vitamin B_6-deficient diet.

2. MATERIALS AND METHODS

Male albino Wistar rats, initial weight 40–50 g, were fed either on the defined, carbohydrate-rich diet, deficient in pyridoxine (B.P. Nutrition, Special Diets Services, Whitfield, Essex) or on that diet to which pyridoxine hydrochloride (Evans Medical, Greenford, Middlesex: 6 mg/kg diet) had been added (the 'supplemented diet'): each rat was allowed up to 25 g of the diet each day and unlimited distilled water. Six pairs were killed after 24 days on the respective diets, one pair after 30 days and one pair after 60 days on the diets. The metatarsals were removed, chilled in *n*-hexane at −70°C, sectioned, with the automatic cutting device at 10 μm in a special bone cryostat (Brights) with the cabinet temperature about −25°C and the tungsten-tipped knife cooled further with solid carbon dioxide packed around its haft [11]. The sections were flash-dried onto glass slides.

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G6PD activity was assayed by the standard quantitative cytochemical procedure [11] in which a particular grade of polyvinyl alcohol (PVA) was used to stabilize the sections and retain 'soluble' dehydrogenase enzymes within the section. This procedure has been extensively validated; in tissue that has a relatively homogeneous cellular content, this procedure yielded G6PD activities comparable to those obtained by conventional biochemical assay [15]. The reaction medium contained: NADP (Boehringer; 3 mM), glucose 6-phosphate disodium salt (Sigma; 5 mM), chloroform-extracted neotetrazolium chloride (Serva; 5 mM), phenazine methosulphate as intermediate electron-carrier (Sigma; 0.7 mM) in a 30% (w/v) solution of PVA (GO4/140; Wacker Chemicals Ltd., Walton, Surrey) in 0.05 M glycyl glycine buffer, pH 8.0. The pH of the final reaction-medium was adjusted, when necessary, to pH 8 before use. The medium was added to the sections and held in position by Perspex rings around the sections. The reactions were done at 37°C in an atmosphere of nitrogen, since oxygen competes with neotetrazolium chloride for reducing equivalents from phenazine methosulphate [16]. In some experiments putrescine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride or ornithine hydrochloride at various concentrations were included in the reaction medium (all from Sigma).

The final reaction-product, an intensely coloured and insoluble precipitate of the formazan (reduced neotetrazolium chloride), was measured solely in the metaphyseal osteoblasts by scanning and integrating microdensitometry [15], at 585 nm [17], with a × 40 objective; scanning spot of 0.4 μm diameter in the plane of the specimen and with a mask that enclosed one cell (so that each measurement was of the amount of activity for a specified time of reaction per cell). These results can be converted to more absolute values if required [18]; the unconverted results were assessed for significance by the Student's *t*-test.

3. RESULTS

3.1. *Conditions of the reaction*

In the control material, the G6PD reaction was linear with time over the first 30 min (fig.1; control). The mean activity (± SE), per cell, measured in 10 serial sections was 19 ± 0.5, giving a reproducibility between sections (coefficient of variance) of ± 8.2%.

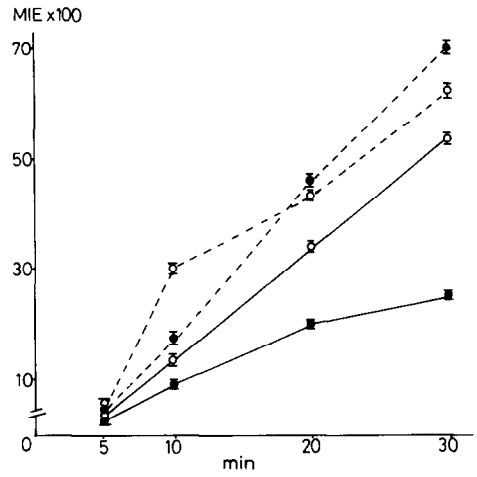


Fig.1. G6PD activity (mean integrated extinction × 100 per cell; bars indicate SE) with various times of reaction, in the absence or presence of putrescine (10^{-3} M) in the osteoblasts of the growth-plate of rats fed either the supplemented (control) or vitamin B_6-deficient diet. (○—○) Control, (○---○) control + putrescine, (●—●) B_6-deficient diet and (●---●) B_6-deficient diet + putrescine.

3.2. *Effect of B_6-deficiency in vivo*

In all the paired specimens, the G6PD activity in the B_6-deficient specimen was less than in the equivalent control specimen (table 1).

3.3. *Effect of putrescine on sections*

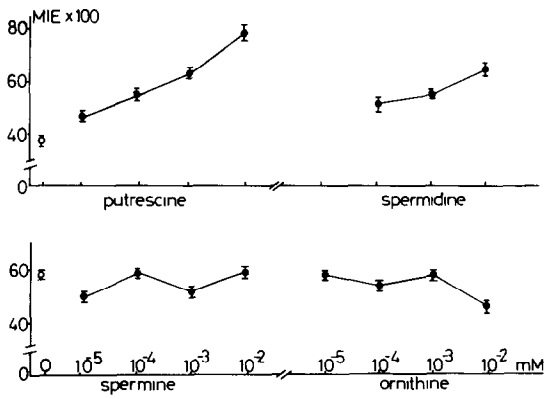
Serial sections were reacted either with the normal reaction-medium or with this medium to which putrescine (10^{-3} M) had been added. The time course of the reaction, over 30 min, in serial sections in which the reaction was stopped at various times, was recorded (fig.1). Frequently this addition of putrescine increased the G6PD activity in the control sections; it generally enhanced that in the sections from B_6-deficient rats up towards that found in the controls (fig.1; table 1). In most specimens, the maximal increase occurred with 20 min reaction time; occasionally 30 min were required.

Table 1

G6PD activity (mean integrated extinction × 100/cell per 20 min reaction), tested either in the presence or absence of putrescine (10^{-3} M) in metaphyseal osteoblasts in sections of the growth-plate of metatarsals of rats fed either the supplemented (control) diet or the same diet deficient in pyridoxine (B_6-deficient diet)

Expt no.	Diet	Days on diet	Body weight (g)	G6PD activity tested		% increase
				Normal medium	Medium + putrescine	
1	control	26	210	48 ± 2.5	53 ± 1.8	10.4
	B_6-deficient	26	134	36 ± 1.4	56 ± 1.8	55.5
2	control	30	211	34 ± 1.8	43 ± 1.8	26.5
	B_6-deficient	30	161	20 ± 1.1	46 ± 1.6	130
3[a]	control	60	296	23 ± 0.9	27 ± 1.1	17.4
	B_6-deficient	60	190	8.0 ± 0.7	20 ± 0.9	150
3[b]	control	60	296	22 ± 1.1	27 ± 1.1	22.7
	B_6-deficient	60	190	7.0 ± 0.2	22 ± 0.7	214
4	control	24	130	74 ± 3.0	79 ± 2.0	6.7
	B_6-deficient	24	80	64 ± 2.0	77 ± 1.0	20.3
5	control	24	110	45 ± 2.0	52 ± 3.0	15.6
	B_6-deficient	24	100	39 ± 1.0	46 ± 3.0	17.9
6	control	24	170	55 ± 2.0	55 ± 2.0	0
	B_6-deficient	24	140	30 ± 2.0	49 ± 3.0	63.3
7	control[a]	24	180	64 ± 2.0	63 ± 2.0	0
	B_6-deficient[a]	24	160	51 ± 3.0	73 ± 3.0	43.1
8	control	24	160	49 ± 2.0	59 ± 3.0	20.4
	B_6-deficient	24	175	38 ± 3.0	53 ± 2.0	39.5

[a,b] Two metatarsals from the same rat
[c] Tested after 30 min reaction



3.4. *Specificity*

No measurable deposition of the reaction product was found when either glucose 6-phosphate or NADP was excluded from the reaction medium; the addition of putrescine to such depleted reaction media had no effect. The increase in G6PD activity induced by putrescine, particularly in the sections from B_6-deficient rats, was dose-dependent (fig.2). Spermidine caused slight stimulation at concentrations of

Fig.2. G6PD activity (mean integrated extinction × 100 per cell for 20 min reaction; bars indicate SE) in sections from B_6-deficient rats tested with and without (○) various concentrations of putrescine, spermidine, spermine and ornithine (●) in the reaction medium.

10^{-4}–10^{-2} M, whereas no stimulation was found with either spermine or ornithine at concentrations of 10^{-5}–10^{-2} M (fig.2). All these tests were done with 20 min reaction times.

4. DISCUSSION

Rats fed on a vitamin B_6-deficient diet had decreased levels of G6PD activity in the osteoblasts of the growth-plate. In other studies on fracture healing in rats fed this B_6-deficient diet, it has been shown that, out of four oxidative enzymes and alkaline phosphatase that were tested, only the G6PD activity was consistently depressed (unpublished). Thus the findings in the present study were in conformity with the possibility that G6PD activity may be regulated by some pyridoxalphosphate related system. The finding that the addition of putrescine to the medium in which the sections were reacted, could restore the depressed G6PD activity to the level found in the control sections, indicates that it may be this product of the pyridoxal phosphate-dependent ornithine decarboxylase activity, that regulates G6PD activity. The mechanism by which such activation can occur, even in sections, awaits elucidation. The special interest in this phenomenon is that it may indicate why elevated ornithine decarboxylase activity is such a frequent concomitant of cellular stimulation, particularly of growth and of such conditions that involve enhanced activity of the pentose-phosphate pathway.

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