Novel functions of vitamin B₆

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Because of its central importance in amino acid metabolism, requirements and reference intakes for vitamin B_6 are usually expressed per g protein intake. However, perhaps only 20% of the total body content of vitamin B_6 is involved as a coenzyme in amino acid metabolism; the greater part is present in muscle as the coenzyme for glycogen phosphorylase (*EC* 2.4.1.1; Coburn *et al.* 1988). These two metabolic functions of the vitamin have been known for many years. Over the last decade or so a novel function of vitamin B_6 has emerged: as a factor modulating the actions of steroid and other hormones that act in the cell nucleus. Deficiency of vitamin B_6 results in increased and prolonged nuclear uptake of steroid hormones and enhanced end-organ sensitivity to hormone action; this may be relevant in the aetiology and treatment of hormone-dependent cancer of the breast, uterus and prostate.

METABOLIC ROLES OF VITAMIN B₆

There are three main areas in which vitamin B_6 is important; in each case pyridoxal 5'-phosphate (PLP) is the metabolically active vitamer:

(1) in reactions of amino acids (and other amino compounds) the carbonyl group of the coenzyme forms a Schiff base (aldimine) with the amino group of the substrate, leading to labilization of bonds around the α-C, followed by decarboxylation to the amine, amino transfer to the oxo-acid or a variety of reactions of the side-chain of the amino acid;

(2) in the reaction of glycogen phosphorylase the coenzyme remains tightly bound through its carbonyl group to a lysine residue in the enzyme and the reactive group is the phosphate, which is ionized in the activated form of the enzyme (but unionized in the inactive form), and acts as a proton shuttle or buffer to facilitate the phosphorolysis of glycogen by inorganic phosphate (Palm *et al.* 1990);

(3) in the modulation of action of steroid (and other nuclear-acting) hormones. The mechanism is not known, but again the carbonyl group seems to be important; PLP acts to release the steroid hormone–receptor complex from DNA binding and terminate hormone action.

THE MODEL OF STEROID HORMONE ACTION

A variety of different hormones are all believed to share a similar mode of action, binding to intracellular receptor proteins, then interacting with transcription factors and hormone-response elements on DNA to change the rate of transcription and, hence, the synthesis of specific proteins. Such hormones include the oestrogens, dihydro-testosterone, progesterone, glucocorticoid and mineralocorticoid steroids, the thyroid hormone tri-iodothyronine and the active metabolites of vitamins D (calcitriol) and A (retinol, retinoic acid and *cis*-retinoic acid), as well as a number of growth factors. The

intracellular receptors for these hormones have a similar structure, including two 'Zn finger' domains (regions of the polypeptide chain forming a finger-like structure, stabilized by a Zn ion). Collectively such receptors are known as Zn-finger proteins, or the steroid hormone super-family of receptors (for a detailed review, see Parker, 1991). It has been by identification of receptor protein in tissues not previously known to be responsive to vitamins A and D that novel functions of the retinoids and calcitriol have been discovered in recent years.

The receptor protein may initially be cytosolic or nuclear; in the absence of the hormone it diffuses readily across the nuclear membrane and can be extracted into the cytosolic fraction on tissue homogenization. On binding the hormone, the receptor undergoes activation, forming a dimer that binds to transcription factors and the hormone-response element of DNA; the activated hormone–receptor complex cannot readily be extracted from the nucleus. The molecular mechanisms involved in releasing the hormone–receptor complex from DNA binding, and so terminating the nuclear action of the hormone, are less well understood; it is here that PLP is believed to be important.

INTERACTIONS OF VITAMIN B_6 WITH STEROID HORMONE RECEPTORS

One of the classical ways of investigating which amino acid side-chains in a protein are functionally important is to react the protein with a specific reagent and investigate the effects on activity. The aldehydes of vitamin B_6, pyridoxal and PLP, have long been used as probes for important lysine residues; not only will the carbonyl group form a Schiff base with an exposed ϵ-amino group of lysine, but this can be reduced (for example, with NaB^3H_4), so as to label covalently the critical lysine residue. Studies of this kind during the 1970s showed that a variety of steroid hormone receptors have a lysine residue that is essential for binding to DNA; it is now known that there is a highly conserved lysine residue in the Zn finger region of all the steroid-hormone-receptor super-family (Parker, 1991).

Cake *et al.* (1978) showed that reaction of glucocorticoid receptor with PLP prevented the binding of the activated hormone–receptor complex to DNA–cellulose *in vitro*. Other workers showed that PLP facilitated the extraction of a variety of hormone–receptor complexes from isolated nuclei, including those for glucocorticoids, oestradiol, androgens and progesterone (for a review, see Bender, 1987). In all cases the effects were specific for PLP – unphosphorylated pyridoxal was ineffective, suggesting a specific binding site on the receptor protein, rather than simply reaction of the carbonyl group with the ϵ-amino group of an exposed lysine residue. Furthermore, the effects were observed at physiologically low concentrations of PLP. These observations led Cidlowski & Thanassi (1981) to propose that PLP may have a physiological role in steroid hormone action, acting to release the hormone–receptor complex from tight nuclear binding, so terminating the nuclear action of the steroid and recycling receptor protein for further uptake of hormone.

EFFECTS OF VITAMIN B_6 DEFICIENCY ON STEROID UPTAKE INTO TARGET TISSUES

DiSorbo *et al.* (1980) showed that in vitamin B_6-deficient rats there was an increase in the liver content of activatable glucocorticoid receptors and an increased rate of steroid translocation into the nucleus *in vitro*.

Holley *et al.* (1983) demonstrated increased uptake of [^{3}H]oestradiol into the nuclear fraction of liver and uterus, and increased uptake into the hypothalamus, in vitamin B$_6$-deficient rats. There was a significant negative correlation between tissue or nuclear uptake of the hormone and indices of vitamin B$_6$ nutritional status. Further studies showed that following injection of the hormone there was a greater and more prolonged nuclear accumulation of [^{3}H]testosterone in the prostate of deficient male rats (Symes *et al.* 1984) and of [^{3}H]oestradiol in the uterus of deficient female rats (Bowden *et al.* 1986) than in control animals maintained on an adequate intake of vitamin B$_6$.

Bender *et al.* (1989) showed that slices of uterus from vitamin B$_6$-deficient rats accumulated more [^{3}H]oestradiol on incubation than did tissue from control animals; when deficient animals were repleted with vitamin B$_6$ 30–60 min before killing, there was a further increase in steroid uptake. Similarly, isolated hepatocytes from vitamin B$_6$-deficient animals accumulated more of the glucocorticoid analogue [^{3}H]dexamethasone than did cells from control animals. Pre-incubation of the isolated cells with PLP again increased the uptake of the steroid.

These results support the hypothesis of Cidlowski & Thanassi (1981) that PLP acts *in vivo* to release hormone–receptor complexes from tight nuclear binding; in deficiency the hormone remains tightly bound in the nucleus for longer, and vitamin repletion releases more receptor protein from nuclear binding.

END-ORGAN RESPONSIVENESS TO HORMONE ACTION IN VITAMIN B$_6$ DEFICIENCY

A number of results suggest that the increased and prolonged nuclear uptake of steroid hormones in target tissues of vitamin B$_6$-deficient animals is functionally important, increasing the sensitivity of the tissue to low levels of hormone stimulation.

Bender *et al.* (1988) showed that in castrated male rats both the rate of increase in prostate weight and the mitotic index in the prostate were greater in vitamin B$_6$-deficient animals than in controls following administration of 1 mg testosterone/d. At a higher dose of testosterone (2·5 mg/d) there was no difference between deficient and control animals. This suggests enhanced end-organ sensitivity to stimulation by low levels of hormone.

Bowden *et al.* (1986) investigated the dose response of ovariectomized female rats to the oestrogen ethynyl-oestradiol. Again they showed increased sensitivity to the hormone in vitamin B$_6$-deficient animals. Control animals showed a linear increase in suppression of luteinizing hormone (LH) secretion in response to graded doses of the hormone, while even at the lowest dose of ethynyl-oestradiol used the deficient animals showed more or less complete suppression of LH secretion. As would be expected, the weight of the uterus increased with increasing dose of ethynyl-oestradiol; again the deficient animals showed a steeper dose response than did controls.

Bowden *et al.* (1986) also investigated the induction by ethynyl-oestradiol of peroxidase (*EC* 1.1.11.7) in the uterus. Induction by low doses of ethynyl-oestradiol (over the range of 1–3 ng/kg body weight) was more marked in deficient animals than in controls, with a significantly steeper dose–response curve. While this suggests enhanced responsiveness to low hormone stimulation, interpretation of the results is confounded by the fact that at higher levels of ethynyl-oestradiol (3–4 ng/kg body weight) there was a considerably greater increase in peroxidase activity, attributed to invasion of the uterus

by eosinophils rather than induction of uterine enzyme; this was the same in both groups of animals.

Animal studies suggest that vitamin B_6 deficiency enhances sensitivity to endogenous steroids, as well as to administered hormones. In their studies of [^{3}H]oestradiol uptake into the nucleus, Bowden *et al*. (1986) investigated animals through the oestrous cycle. They found that a greater proportion of the vitamin B_6-deficient animals were in metoestrus and anoestrus, with correspondingly fewer in proestrus and oestrus than expected on the basis of the vitamin B_6-adequate controls.

Symes *et al*. (1984) showed that in vitamin B_6-deficient male rats the plasma concentration of testosterone was only 25% of that in vitamin B_6-adequate control animals. The mechanism for this has not been elucidated. However, despite the abnormally low circulating concentration of testosterone, the deficient animals did not have an elevated plasma concentration of LH, suggesting increased sensitivity of the hypothalamic–pituitary axis to low levels of testosterone. Similarly, there was no difference in the weight of the prostate gland, despite the lower concentration of testosterone, again suggesting increased sensitivity to hormone action.

Dakshinamurti & Lal (1992) showed significant hypertension in vitamin B_6-deficient rats compared with controls receiving an adequate intake of the vitamin, which was normalized within 24 h of a single dose of 10 mg pyridoxine/kg body weight. They interpreted their results as being due to changes in the turnover of three central nervous system neurotransmitters that are synthesized by PLP-dependent reactions, 5-hydroxytryptamine, noradrenaline and γ-aminobutyrate. It is possible, however, that the hypertension was due to increased end-organ sensitivity to aldosterone, a nuclear-acting steroid hormone.

VITAMIN B_6 STATUS AND HORMONE-DEPENDENT GENE EXPRESSION

In cells in culture, manipulation of intracellular PLP affects the expression of steroid-hormone-responsive genes in the way that is predicted by the hypothesis of Cidlowski & Thanassi (1981). Deficiency results in increased induction, while supplementation reduces responsiveness to the hormone.

Allgood and coworkers (Allgood *et al*. 1990; Allgood & Cidlowski, 1992) transfected gene constructs of a hormone-response element and chloramphenicol acetyltransferase (*EC* 2.3.1.28; CAT) as a 'reporter' gene into various cell types in culture. The systems they used were: the human or mouse glucocorticoid response element – CAT gene construct transfected into HeLa cells, E-8.2 mouse fibroblast and T47D human breast cancer cell lines; androgen response element – CAT in mouse fibroblast cells; progesterone response element – CAT in human breast cancer and oestrogen response element – CAT in HeLa cells. Cells were cultured with a normal amount of pyridoxine in the culture medium (the control incubations), with 4-deoxypyridoxine to deplete PLP or in the presence of 1–3 mmol pyridoxine/l (depending on cell type) to increase intracellular PLP. They were then exposed to the appropriate hormone and the activity of CAT determined as an index of enzyme induction and, hence, responsiveness to the hormone. In deficient cells the mean activity of CAT was 1·85-fold that seen in control cells (range for different systems 1·56–2·26); in cells supplemented with vitamin B_6 the mean activity of CAT was 0·56-fold that in control cells (range for different systems 0·32–0·74).

POSSIBLE RELEVANCE TO HUMAN VITAMIN B$_6$ NUTRITION

Clinical disease due to deficiency of vitamin B$_6$ is unknown in human beings, apart from one outbreak among infants during the 1950s, associated with infant formula that had been heat sterilized, so that most of the vitamin was present as the pyridoxyl-lysine adduct, which is both biologically unavailable and may have anti-vitamin activity (Coursin, 1954; Gregory, 1980*a,b*).

Average intakes of vitamin B$_6$ appear to give little cause for concern. Calculations based on data in the *Dietary and Nutritional Survey of British Adults* (Gregory *et al.* 1990) suggest that the 95% range of intakes is 25·5–34·6 µg/g protein for men and 22·4–28·4 µg/g protein for women, compared with a reference nutrient intake of 15 µg/g protein. However, up to half the vitamin B$_6$ in plant foods, which are apparently good sources, is present as pyridoxine glucoside, which has low biological availability. Overall it is estimated that only about 70–80% of the vitamin in the average mixed diet is available (Tarr *et al.* 1981), and presumably considerably less in vegetarian diets.

Unfortunately the *Dietary and Nutritional Survey of British Adults* (Gregory *et al.* 1990) did not include biochemical assessment of vitamin B$_6$ status. A number of studies suggest that between 9 and 30% of the population show biochemical evidence of marginal deficiency or inadequacy as assessed by either plasma concentration of PLP or the activation of erythrocyte alanine (*EC* 2.6.1.12) or aspartate (*EC* 2.6.1.1) aminotransferase by PLP added *in vitro* (Bender, 1989). These results must be interpreted with caution; when both indices have been assessed in the same subjects there is poor concordance, and very few subjects appear to be deficient by both criteria (Bender, 1993).

It is tempting to speculate that vitamin B$_6$ inadequacy may be a factor in the aetiology of hormone-dependent cancer of the breast, uterus and prostate, and in hypertension; conditions where enhanced responsiveness of the target tissue to normal or even lower than normal levels of hormones may be important. There is, as yet, no evidence, although one study has reported that the prognosis in women with breast cancer is inversely correlated with vitamin B$_6$ nutritional status (Bell, 1980). Equally, it may be that high intakes of vitamin B$_6$ will, by attenuating hormone responsiveness, prove to be beneficial in preventing or treating these conditions. Again there is no evidence. Although a considerable body of anecdotal evidence suggests that supplements of vitamin B$_6$ alleviate the symptoms of the premenstrual syndrome, the results of controlled trials are inconclusive (Gunn, 1985).

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