REGULATION OF THE SYNTHESIS OF 1,25-DIHYDROXYVITAMIN D₃ AND 24,25-DIHYDROXYVITAMIN D₃ IN KIDNEY CELL CULTURE

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1. INTRODUCTION

The major circulating form of vitamin D, 25-hydroxyvitamin D₃, is synthesized in the liver and serves as a pool of precursor for the dihydroxylated metabolites, 1,25(OH)₂D₃ and 24,25(OH)₂D₃. 1,25(OH)₂D₃ undoubtedly mediates the primary biological actions classically attributed to vitamin D in the intestine and bone. Although there has accumulated some evidence for a biological role for 24,25(OH)₂D₃ (1-4), the precise nature of this has not yet been elucidated. The kidney is generally considered to be the major site of synthesis of the dihydroxylated metabolites of vitamin D, although both can also be produced by extrarenal tissues such as the placenta (5,6) or by cells derived from extrarenal tissues such as bone (7,8).

In the past decade a great deal of attention has been focused upon the regulation of the production of 1,25(OH)₂D₃ and 24,25(OH)₂D₃. The results of many studies carried out either partially or entirely in vivo have implicated a number of factors in this regulation. These include 1,25(OH)₂D₃, parathyroid hormone, calcitonin, serum and tissue mineral levels of calcium and phosphorus, other steroid hormones such as estrogen and glucocorticoids, pituitary hormones including prolactin and growth hormone, and insulin. Much of this work has been the subject of a recent excellent review (9) and will not be dealt with in detail here.

In this chapter the focus will be upon studies carried out with whole kidney cell preparations in vitro, the aim of which has been to delineate those regulatory factors which exert their effects on 25-OH-D₃ metabolism by direct interaction with the renal cell. In considering possible mechanisms of regulation of the 1-hydroxylase and 24-hydroxylase it should be kept in mind that both enzymes are mitochondrial and that the 1-hydroxylase, at least, is a cytochrome P-450 linked enzyme (10,11) which is coupled to the electron transporting proteins, ferredoxin and ferredoxin reductase.
Thus any change in enzyme activity brought about by an extracellular signal must eventually be explainable in terms of a change in activity of this compartmentalized mitochondrial system.

2. 1,25-DIHYDROXYVITAMIN D₃

From the earliest studies on the renal production of 1,25(OH)₂D₃ and its regulation it has been recognized that maximal 1-hydroxylase activity is observed in preparations obtained from vitamin D deficient animals and that treatment, in vivo, with vitamin D₃ or 1,25(OH)₂D₃ suppresses 1-hydroxylase activity and induces 24-hydroxylase activity (13-16). With the availability of primary cultures of chick kidney cells it was possible to test whether the effect of 1,25(OH)₂D₃ is a direct one on the renal cell or whether it represents a secondary effect, for example a modulation of serum calcium and/or parathyroid hormone levels. There is now ample evidence that 1,25(OH)₂D₃ modulates the metabolism of 25-OH-D₃ through direct action in the kidney cell (17-21).

A time course of this effect is illustrated in Figure 1, which was

![Figure 1. Effect of 1,25(OH)₂D₃ at 10⁻⁷ M (●) or 10⁻⁸ (○) on 1-hydroxylase or 24-hydroxylase activity in chick kidney cell cultures. Following incubation of cultures in serum-free medium for the indicated time, ³H-25-OH-D₃ was added for another 30 minutes. The metabolites of ³H-25-OH-D₃ in this and all other experiments were extracted and separated by high pressure liquid chromatography.]