The role of reversible phosphorylation in the hormonal control of phenylalanine hydroxylase in isolated rat proximal kidney tubules

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Reversible phosphorylation is the major mechanism underlying the short-term hormonal control of phenylalanine hydroxylase activity in the liver. We report here, for the first time, the impact of a range of hormonal effectors on both the phosphorylation state and enzymic activity of phenylalanine hydroxylase present in isolated rat proximal kidney tubules. The most potent stimu-

lator of enzyme phosphorylation was found to be parathyroid hormone, which is known to stimulate the production of cyclic AMP in proximal-tubule cells. In addition, adrenergic amines also stimulated enzyme phosphorylation, although to a lesser extent, through interaction with a mixed α_1 and β receptor population.

INTRODUCTION

The initial step of phenylalanine degradation in man and other mammals involves hydroxylation to tyrosine. This reaction is catalysed by the enzyme phenylalanine hydroxylase [L-phenylalanine:tetrahydropteridine:oxygen oxidoreductase (4-hydroxylating); EC 1.14.16.1]:

This reaction is generally held to be the key regulatory step in the degradation of phenylalanine (Kaufman, 1971). The major site of phenylalanine hydroxylation is the liver; however, there is also appreciable phenylalanine hydroxylase activity in kidney (Berry et al., 1972; McGee et al., 1972; Ayling et al., 1975). The purification of phenylalanine hydroxylase from rat liver and kidney cortex has indicated, on the basis of immunological, kinetic and a number of physical properties, a basic similarity between the two enzymes (Ayling et al., 1974; Rao and Kaufman, 1986).

The hepatic enzyme is controlled, in the short term, by a phosphorylation–dephosphorylation process (Donlon and Kaufman, 1978; Fisher and Pogson, 1984). Glucagon and α -adrenergic agents stimulate the phosphorylation of the hepatic enzyme through the action of the cyclic AMP-dependent protein kinase and a Ca^{2+} /calmodulin-dependent protein kinase respectively (Doskeland et al., 1984, 1992). Changes in phosphorylation state of the enzyme are closely associated with alterations in the tetrahydrobiopterin-dependent enzymic activity (Abita et al., 1976). We have recently shown that the enzyme purified from kidney is a substrate for cyclic AMP-dependent phosphorylation *in vitro* (Richardson et al., 1991). In addition, we have also been able to demonstrate that the phosphorylation state of the enzyme in isolated rat kidney tubules can be altered by incubation in the presence of dibutyryl cyclic AMP (Green et al., 1990). These observations raise the possibility that the state of phosphorylation of renal phenylalanine hydroxylase *in vivo* is under hormonal control and that alterations in the phosphorylation state may be an important contributory factor in the physiological control of phenylalanine hydroxylase activity.

The studies described in the present paper were undertaken to clarify the role of hormonal modulation of phosphorylation state in the control of phenylalanine hydroxylase from isolated rat kidney tubules.

MATERIALS AND METHODS

Chemicals

Reagents and radiochemicals were obtained from the sources given previously (Green et al., 1990), with the following additions. Peptide hormones, adrenergic effectors and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma Chemical Co., Poole, Dorset, U.K. Alkaline phosphatase-labelled rabbit anti-sheep IgG was obtained from ICN Biomedicals, High Wycombe, Bucks., U.K. Alkaline phosphatase-labelled goat anti-mouse IgG and the alkaline phosphatase-conjugate substrate kit were obtained from Bio-Rad, Hemel Hempstead, Herts., U.K. Okadaic acid was from Gibco BRL, Paisley, Scotland, U.K. Other chemicals were obtained from BDH Chemicals, Poole, Dorset, U.K. Tetrahydrobiopterin was a gift from Dr. C. I. Pogson, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, U.K. Monoclonal antibody PH 7 was a gift from Dr. R. G. H. Cotton, Murdoch Institute, Royal Children's Hospital, Melbourne, Victoria, Australia.

Animals

Male Wistar rats (University of Liverpool breeding colony) weighing 180–220 g were used throughout. Animals were fed *ad libitum* [Labsure Animal Diet (CRM); C. Hill Group, Poole, Dorset, U.K.].

Preparation and incubation of proximal kidney tubules

Proximal kidney tubules were prepared by collagenase digestion essentially as described by Askin et al. (1990). Portions (2.0 ml) of the final tubule suspension were incubated at 37 °C with shaking (100 cycles/min) in Krebs–Henseleit (1932) medium. Experiments involving the use of [^{32}P]P_i were carried out in low-phosphate (0.04 mM) Krebs–Henseleit medium as described

previously (Fisher and Pogson, 1984). In all cases, the incubation medium was supplemented with 2.5 mM CaCl_2 , 2% (w/v) BSA and lactate/pyruvate (9:1; final concn. 10 mM). Where appropriate, hormones and other effectors were added after incubation for 60–90 min, at which time the ^{32}P content of phenylalanine hydroxylase had attained a steady state.

Unless otherwise stated, the isolated tubules were incubated in the presence of effectors for 2 min. Incubations were terminated by dilution with a 4-fold excess of ice-cold incubation medium. Cells were separated from the medium by centrifugation at 500 g for 30 s at room temperature and then resuspended in a buffer containing 250 mM sucrose, 20 mM Tris/HCl, 2 mM sodium phosphate, 5 mM EDTA, 100 mM NaF, 0.5 mM phenylmethanesulphonyl fluoride, leupeptin (20 $\mu\text{g}/\text{ml}$), pepstatin (2 $\mu\text{g}/\text{ml}$) and chymostatin (2 $\mu\text{g}/\text{ml}$), pH 7.4. Cells were broken by three cycles of freezing in liquid N_2 and thawing in water at 37 °C. The final extracts were centrifuged at 12000 g for 2 min at room temperature before enzyme assay or SDS/PAGE analysis.

Viability of tubule incubations

In all experiments, metabolic integrity was assessed by measurement of cellular ATP content (Dickson and Pogson, 1977). Kidney tubule preparations had an ATP content in excess of 6.0 nmol/mg dry wt. During the course of incubations (up to 2 h) there was no significant decrease in ATP content. In addition, the gluconeogenic capacity of kidney tubule preparations was also monitored. The basal gluconeogenic rate, from 10 mM lactate, was 99 ± 8 nmol/h per mg dry wt. (mean $\pm$ S.E.M. for 16 different preparations). This gluconeogenic activity was stimulated by a range of effectors [100 μM dibutyryl cyclic AMP, 146 ± 16 ; 1 $\mu\text{g}/\text{ml}$ parathyroid hormone (PTH), 155 ± 7 ; 10 μM phenylephrine, 153 ± 9 (as % of the basal gluconeogenic rate from 10 mM lactate; means $\pm$ S.E.M. for at least three different tubule preparations)].

Immunoblot analysis of phenylalanine hydroxylase

Tubule-cell extracts were subjected to SDS/PAGE (Laemmli, 1970) and then transferred to nitrocellulose filters (Towbin et al., 1979). Immunoblotting was performed with a sheep antiserum to rat liver phenylalanine hydroxylase (Fisher and Pogson, 1984), or alternatively, the monoclonal antibody PH 7 (Smith et al., 1987). Immunoblotting with the polyclonal antibody provided an estimate of the total amount of phenylalanine hydroxylase protein present in the sample. Immunoblotting with the monoclonal antibody PH 7, which specifically recognizes the phosphorylated form of the enzyme (Smith et al., 1987), provided an indication of the phosphorylation state of the hydroxylase in the sample (Green et al., 1990). Samples of each extract were probed with both PH 7 and the polyclonal antibody, so that the ratio of phosphorylated enzyme to total enzyme could be established, as described by Green et al. (1990).

Antibody binding was revealed by a modification of the method described previously (Green et al., 1990). In the present experiments, immunoblots were developed by incubation with 3 μl of alkaline phosphatase-labelled rabbit anti-sheep IgG or alkaline phosphatase-labelled goat anti-mouse IgG, as appropriate. This was followed by incubation in 15 ml of 100 mM NaHCO_3 , pH 9.8, to which was added 4.5 mg of 5-bromo-4-chloro-3-indolyl phosphate and 2.25 mg of Nitro Blue Tetrazolium. Immunoreactive protein was quantified by densitometric scanning of immunoblots at 500 nm by using a Zeiss chromato-

graphic scanner. A linear relationship between A_{500} and protein load was verified for each blot. In addition, a linear relationship between the A_{500} and the extent of phosphorylation of the enzyme was observed for PH 7 immunoblots.

Analysis of [^{32}P]P_i incorporation into phenylalanine hydroxylase

^{32}P -labelled phenylalanine hydroxylase was immunoprecipitated from tubule-cell extracts, by using the polyclonal antibody, as described previously (Fisher and Pogson, 1984). The resulting immunoprecipitates were then subjected to SDS/PAGE, and the distribution of radioactivity within the dried gels was assessed by autoradiography using Fuji (RX) X-ray film and intensifying screens.

Other methods

Phenylalanine hydroxylase was assayed, in the presence of tetrahydrobiopterin (Hasegawa and Kaufman, 1982), by a fluorimetric technique for the determination of tyrosine formation (Waalkes and Udenfriend, 1957).

RESULTS AND DISCUSSION

Protein kinase and protein phosphatase effectors

Figure 1 and Table 1 show the impact, on phenylalanine hydroxylase phosphorylation state, of incubation of proximal kidney tubules in the presence of a range of effectors. These data show that dibutyryl cyclic AMP caused a significant stimulation of the phosphorylation of phenylalanine hydroxylase, as determined both by the PH 7/polyclonal-antibody binding ratio and autoradiographic analysis of ^{32}P labelling. This is consistent with our previous observation that the enzyme in isolated kidney tubules shows increased incorporation of [^{32}P]P_i after incubation with dibutyryl cyclic AMP (Green et al., 1990). The alteration in

Figure 1 Autoradiographic analysis of kidney tubule extracts

Autoradiographs were obtained from ^{32}P -labelled isolated tubule extracts subjected to SDS/PAGE as described in the Materials and methods section. A representative autoradiograph from several such experiments is shown here. Lane 1, no additions; lane 2, 100 μM dibutyryl cyclic AMP; lane 3, 0.025% (v/v) dimethyl sulphoxide (DMSO) only; lane 4, 1 $\mu\text{g}/\text{ml}$ PMA in 0.025% DMSO; lane 5, 1 μM okadaic acid in 0.025% DMSO. The relative mobilities of a series of molecular-mass markers, after SDS/PAGE, are indicated on the left of the autoradiograph: A, BSA (66 kDa); B, egg albumin (45 kDa); C, glyceraldehyde-3-phosphate dehydrogenase (36 kDa); D, carbonic anhydrase (29 kDa).

Table 1 Impact of protein kinase and protein phosphatase effectors on kidney tubule phenylalanine hydroxylase

The phosphorylation state of phenylalanine hydroxylase in kidney tubule extracts was assessed by analysis of the PH 7 monoclonal-antibody/polyclonal-antibody binding ratio as described in the Materials and methods section. Results are expressed as means $\pm$ S.E.M. for tubule preparations from at least three different animals. The significance of differences between means was assessed by a paired *t* test: **P* (versus basal enzyme phosphorylation) < 0.05. Other differences not significant.

Addition	Enzyme phosphorylation (% of basal)
None	100
100 μ M Dibutyryl cyclic AMP	213 $\pm$ 10*
0.025% DMSO	111 $\pm$ 12
PMA (1 μ g/ml in 0.025% DMSO)	111 $\pm$ 3
1 μ M Okadaic acid (in 0.025% DMSO)	171 $\pm$ 11*

the phosphorylation state of phenylalanine hydroxylase was associated with an increase in the tetrahydrobiopterin-dependent enzymic activity [no additions, 0.51 ± 0.05 ; 100 μ M dibutyryl cyclic AMP, 1.08 ± 0.07 (nmol/min per mg of protein; means $\pm$ S.E.M. for at least three different tubule preparations; *P* < 0.05)].

Incubation of proximal tubules with okadaic acid, a potent protein phosphatase 1 and 2A inhibitor (Hardie et al., 1991), also brought about an increase in hydroxylase phosphorylation (see Table 1 and Figure 1). These alterations are of a similar magnitude to those which we, and others, have observed after incubation of isolated liver cells with okadaic acid (Green and Fisher, 1991; Doskeland et al., 1992) and are consistent with the suggestion that the major protein phosphatase activity responsible for the dephosphorylation of phenylalanine hydroxylase, under physiological conditions, is protein phosphatase 2A (Pelech et al., 1984). It should be noted that an alternative explanation for the impact of okadaic acid on phenylalanine hydroxylase phosphorylation state has recently been advanced (Doskeland et al., 1992). These workers have suggested that okadaic acid may exert its effect on phenylalanine hydroxylase phosphorylation state indirectly, through the phosphorylation of a Ca^{2+} /calmodulin-dependent protein kinase. This protein kinase is a substrate for okadaic acid-sensitive protein phosphatase(s); therefore, incubation in the presence of okadaic acid may lead to the autophosphorylation and activation of this enzyme. The activated protein kinase may then phosphorylate phenylalanine hydroxylase. The contribution of such a mechanism to the okadaic acid-dependent phosphorylation of kidney tubule phenylalanine hydroxylase remains to be established; however, the presence of Ca^{2+} /calmodulin-dependent protein kinase(s) in renal tissues has been noted by other workers (Fukunaga et al., 1988; Hanley et al., 1990).

Table 1 and Figure 1 show that the protein kinase C activator PMA did not bring about significant alterations in the phosphorylation state of the enzyme. In addition, no significant alteration in tetrahydrobiopterin-dependent enzymic activity was observed after incubation of isolated tubules with PMA (results not shown). This is consistent with the lack of any characteristic protein kinase C recognition motifs in the rat phenylalanine hydroxylase polypeptide sequence (Dahl and Mercer, 1986) and with previous observations that the phosphorylation state of the enzyme, from isolated liver cells incubated in the presence of PMA, is not significantly increased (Fisher et al., 1986).

Table 2 Impact of peptide hormones on kidney tubule phenylalanine hydroxylase

The phosphorylation state of phenylalanine hydroxylase in kidney tubule extracts was assessed by analysis of the PH 7 monoclonal-antibody/polyclonal-antibody binding ratio as described in the Materials and methods section. Results are expressed as means $\pm$ S.E.M. for tubule preparations from at least three different animals. The significance of differences between means was assessed by a paired *t* test: **P* (versus basal enzyme phosphorylation) < 0.05. Other differences not significant.

Addition	Enzyme phosphorylation (% of basal)
None	100
PTH (1 μ g/ml)	226 $\pm$ 13*
0.1 μ M Glucagon	108 $\pm$ 7
0.1 μ M Insulin	105 $\pm$ 5
0.1 μ M Vasopressin	72 $\pm$ 11
0.1 μ M Angiotensin	78 $\pm$ 10

Peptide hormones

Table 2 shows the impact of a variety of peptide hormones on kidney tubule phenylalanine hydroxylase phosphorylation state, as determined by analysis of the PH 7/polyclonal-antibody binding ratio of cell extracts. The only effector in this category to have a significant effect was parathyroid hormone (PTH), or PTH fragment 1–34 (results not shown). This stimulation of phosphorylation was associated with a significant increase in the tetrahydrobiopterin-dependent phenylalanine hydroxylase activity in tubule-cell extracts [no additions, 0.41 ± 0.06 ; 1 μ g/ml PTH, 1.16 ± 0.03 (nmol/min per mg of protein; means $\pm$ S.E.M. for at least three different tubule preparations; *P* < 0.05)].

Further experiments revealed a PTH-concentration-dependent increase in the phosphorylation of the enzyme isolated from tubule cell extracts. Figure 2(a) shows a typical PH 7 immunoblot analysis of the effect of a range of concentrations of PTH on kidney tubule phenylalanine hydroxylase phosphorylation state. Figure 2(b) shows an autoradiographic analysis of phenylalanine hydroxylase immunoprecipitated from proximal tubules incubated in the presence of [32 P]P_i and the same range of concentrations of PTH. Both experimental approaches indicated a similar relationship between PTH concentration and the extent of enzyme phosphorylation, with a half-maximally effective concentration of PTH of approx. 6 ng/ml, as illustrated in Figure 2(c). This indicates that concentrations of PTH in the physiological range (0.2–2 ng/ml; Agus et al., 1981) can bring about alterations in the phosphorylation state of renal phenylalanine hydroxylase.

The stimulation of enzyme phosphorylation by PTH is consistent with the ability of this hormone to stimulate cyclic AMP production in rat proximal-tubule cells (Sundaresan et al., 1987). Indeed, PTH-dependent stimulation of adenylate cyclase has been used as a marker for proximal tubules (Morel et al., 1981). In contrast, although glucagon and insulin receptors have been identified in certain regions of the rat kidney tubule, they appear to be absent, in the case of glucagon (Butlen and Morel, 1985), or of relatively low abundance, in the case of insulin (Pillion et al., 1988), in the proximal tubule. This may explain the inability of these hormones to promote any alterations in phenylalanine hydroxylase phosphorylation state in the present experiments (see Table 2). It would also explain the lack of effect of glucagon injection *in vivo* on the phosphorylation state of kidney phenyl-

Figure 2 Effect of different concentrations of PTH on phenylalanine hydroxylase phosphorylation in isolated kidney tubules

(a) Immunoblots were obtained from isolated kidney tubules incubated in the presence of a range of concentrations of PTH. Tubule extracts were subjected to SDS/PAGE, transferred to nitrocellulose and immunostained with monoclonal antibody PH 7 as described in the Materials and methods section. A representative immunoblot from three such experiments is shown here. Lane C, no additions; lanes 4 to -1, tubules incubated in the presence of 10^{-4} to 10^{-1} μg/ml PTH respectively. (b) Autoradiographs were obtained from 32 P-labelled isolated tubules incubated in the presence of a range of concentrations of PTH. Tubule extracts were subjected to SDS/PAGE as described in the Materials and methods section. A representative autoradiograph from three such experiments is shown here. Lane C, no additions; lanes 5 to -1, tubules incubated in the presence of 10^{-5} – 10^{-1} μg/ml PTH respectively. In (a) and (b), the relative mobilities of a series of molecular-mass markers, after SDS/PAGE, are indicated on the left: A, BSA (66 kDa); B, egg albumin (45 kDa); C, glyceraldehyde-3-phosphate dehydrogenase (36 kDa); D, carbonic anhydrase (29 kDa); E, trypsinogen (24 kDa). (c) The relationship between PTH concentration and the extent of phenylalanine hydroxylase phosphorylation was assessed by analysis of the PH 7 monoclonal-antibody/polyclonal-antibody binding ratio as described in the Materials and methods section. Results are expressed as means $\pm$ S.E.M. for tubule preparations from at least three different animals.

alanine hydroxylase, reported by other workers (Rao and Kaufman, 1986).

Table 2 also shows that angiotensin II and vasopressin have

Figure 3 Effect of adrenergic agents on phenylalanine hydroxylase phosphorylation state in isolated kidney tubules

The effect of a range of adrenergic agonists and antagonists (A, adrenaline; Z, prazosin; Y, yohimbine; P, propranolol; N, noradrenaline; PE, phenylephrine; I, isoprenaline) on the extent of phenylalanine hydroxylase phosphorylation, in isolated kidney tubules, was assessed by analysis of the PH 7 monoclonal-antibody/polyclonal-antibody binding ratio as described in the Materials and methods section. Each effector was present at a final concentration of 10^{-6} M. Where appropriate, isolated tubules were preincubated for 5 min in the presence of antagonist before the addition of agonist. Results are expressed as means $\pm$ S.E.M. for tubule preparations from at least three different animals. The significance of differences between means was assessed by a paired *t* test: **P* (agonist only versus agonist with antagonist) < 0.05 . In each case *P* (agonist only versus no treatment) < 0.05 . Other differences not significant.

little effect on phenylalanine hydroxylase phosphorylation state in isolated proximal tubules. This, again, may reflect the constitution of the respective receptor populations in the proximal tubule. The angiotensin II receptors found in the rat proximal tubule are predominantly of the B subtype, which is linked, in an inhibitory fashion, to adenylate cyclase (Douglas, 1987; Sekar et al., 1990). Although most of the vasopressin receptors in the kidney are of the V_2 subtype, which stimulate adenylate cyclase activity and are involved in the anti-diuretic action of this hormone, these receptors are largely absent from the rat proximal tubule (Sunderesan et al., 1987; Jung and Endou, 1991).

Adrenergic amines

The impact of a variety of adrenergic agents on the phosphorylation state of phenylalanine hydroxylase in isolated proximal tubules is shown in Figure 3. The rat proximal kidney tubule is believed to contain a mixed population of α_1 , α_2 and β receptors (Jacobs and Chan, 1986; Umemura et al., 1986; Sunderesan et al., 1987; Stanko et al., 1990). Receptors of the α_1 and α_2 classes are believed to predominate over the β receptor class in the rat proximal tubule. This is consistent with the data shown in Figure 3. These indicate that incubation of proximal tubules with either phenylephrine (an α_1 -specific agonist) or isoprenaline (a β -specific agonist) caused stimulation of phenylalanine hydroxylase phosphorylation. Stimulation of tetrahydrobiopterin-dependent enzymic activity was also seen after incubation of isolated tubules with either the α_1 -agonist or the β -agonist [no additions, 0.46 ± 0.04 ; 10^{-6} M phenylephrine, 0.74 ± 0.06 ; 10^{-6} M isoprenaline, 0.75 ± 0.04 (nmol/min per mg of protein; means $\pm$ S.E.M. for at least three different tubule preparations; $P < 0.05$)]. Although the increase in phosphorylation associated with the α_1 -agonist appeared to be somewhat greater than that observed with the β -agonist, the

increases in enzymic activity associated with these agonists were not significantly different. In either case, the alteration in both phosphorylation state and enzymic activity was less substantial than that observed with maximal concentrations of PTH (see above).

Figure 3 shows that both adrenaline and noradrenaline were able to stimulate phenylalanine hydroxylase phosphorylation in isolated tubules. In addition, prazosin (an α_1 -specific antagonist) is significantly more effective than either propranolol (a β -specific antagonist) or yohimbine (an α_2 -specific antagonist) at diminishing either the adrenaline- or noradrenaline-stimulated phosphorylation of phenylalanine hydroxylase in isolated kidney tubules. These observations suggest that physiological adrenergic stimulation of phenylalanine hydroxylase in the rat proximal kidney tubule may, as in the liver cell (see the Introduction), be largely mediated by α_1 -adrenergic receptors, with a relatively small contribution from β -adrenergic receptors.

General discussion

The contribution of hormonally stimulated alterations in hepatic phenylalanine hydroxylase phosphorylation state to the overall control of phenylalanine degradation *in vivo* has been appreciated for some time (Donlon and Kaufman, 1978). In contrast, the possible role of reversible phosphorylation in the regulation of the renal enzyme has received little attention. Although the work of Rao and Kaufman (1986) indicated that the enzyme purified from renal tissues was a phosphoprotein, we have only recently been able to demonstrate the cyclic AMP-dependent phosphorylation of the purified renal enzyme (Richardson et al., 1991).

Here we have shown that the enzyme present in preparations of isolated rat kidney tubule cells is subject to hormonally controlled alterations in phosphorylation state. Our results indicate that the most significant effectors in this respect are PTH and the adrenergic amines. This reflects to a large extent the hormone receptor populations known to be present in the rat proximal tubule. PTH exerts its effects on proximal-tubular-cell metabolism through increases in the intracellular concentration of cyclic AMP and consequent activation of the cyclic AMP-dependent protein kinase (Agus et al., 1981). In this way, PTH is able to stimulate proximal-tubule gluconeogenesis. In this respect it should be remembered that the degradation of phenylalanine results in formation of the gluconeogenic precursor fumaric acid. The PTH-stimulated phosphorylation of phenylalanine hydroxylase in tubular cells may therefore contribute to the provision of an increased supply of gluconeogenic precursors.

The present results indicate that adrenergic amines, acting through a mixed population of α_1 and β receptors, stimulate the phosphorylation of phenylalanine hydroxylase in isolated proximal tubules. It may be expected that occupation of β -adrenergic receptors leads to the activation of adenylate cyclase and subsequent activation of the cyclic AMP-dependent protein kinase. In contrast, occupation of α_1 -receptors may lead to polyphosphoinositide turnover and the subsequent mobilization of intracellular stores of Ca^{2+} (Downes and MacPhee, 1990). As has been discussed above, Ca^{2+} /calmodulin-dependent protein kinase(s) have been identified in renal tissues and may therefore mediate the α_1 -adrenergic stimulation of the phosphorylation of phenylalanine hydroxylase. In this context it is of interest that we have previously failed to demonstrate significant alterations in proximal-tubule phenylalanine hydroxylase phosphorylation state, as measured by incorporation of [^{32}P]P_i, following incubation in the presence of the Ca^{2+} ionophore A23187 (Green et al., 1990). This contrasts with the A23187-dependent

phosphorylation of phenylalanine hydroxylase in isolated liver cells previously observed by ourselves (Fisher et al., 1984) and other workers (Garrison, 1983). This difference in responsiveness may reflect distinct tissue sensitivities to ionophore-mediated Ca^{2+} influx. For example, in contrast with liver, renal tissue contains an abundance of Ca^{2+} -dependent proteinases (Hayashi et al., 1987; Kawashima et al., 1988; Blomgren et al., 1989), which may be rapidly activated after exposure to A23187. This notwithstanding, the mechanism underlying the α_1 -adrenergic stimulation of renal phenylalanine hydroxylase phosphorylation requires further investigation.

The present results show that hormonally stimulated phosphorylation of phenylalanine hydroxylase in isolated kidney tubules is associated with a significant increase in phenylalanine hydroxylase activity in tubule cell extracts. It remains to be demonstrated that such alterations in activity are reflected in increased phenylalanine hydroxylation flux *in vivo*. Our previous experiments (Askin et al., 1990) have indicated that incubation of isolated proximal tubules in the presence of dibutyryl cyclic AMP can lead to the stimulation of phenylalanine hydroxylation flux. However, the responsiveness of renal phenylalanine hydroxylation flux to this effector appears to be quite distinct from that observed in liver cells (Askin et al., 1990). The precise relationship between the hormonal stimulation of enzyme phosphorylation and alterations in phenylalanine hydroxylation flux in isolated proximal tubules therefore deserves further investigation.

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