The effects of various anions and cations on the regulation of pyruvate dehydrogenase complex activity from pig kidney cortex

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The activity of pyruvate dehydrogenase complex (PDC) purified from pig kidney cortex was found to be affected by various uni- and bi-valent ions. At a constant ionic strength of 0.13 M at pH 7.8, K^+ , Na^+ , Cl^- , HCO_3^- and HPO_4^{2-} had significant effects on the activity of PDC: Na^+ , K^+ and HPO_4^{2-} stimulated, but HCO_3^- and Cl^- inhibited. The stimulatory effect of Na^+ was mediated by a change in the V_{max} of PDC only, whereas K^+ produced an increase in V_{max} and a change in the Hill coefficient (h). The extent of stimulation produced by HPO_4^{2-} on the activity of PDC was dependent on the concentrations of K^+ and Na^+ . Both cations at concentrations higher than 40 mM partially prevented the effect of HPO_4^{2-} . Cl^- and HCO_3^- anions decreased the V_{max} of the enzyme and increased the $s_{0.5}$ for pyruvate. The effects of Na^+ , K^+ , Cl^- , HPO_4^{2-} and HCO_3^- on the activity of PDC were additive. In the presence of 80 mM- K^+ , 20 mM- Na^+ , 10 mM- HPO_4^{2-} , 20 mM- Cl^- and 20 mM- HCO_3^- the activity of PDC was increased by 30 %, the $s_{0.5}$ for pyruvate was increased from 75 to 158 μ M and h was decreased from 1.3 to 1.1. Under these conditions and at 1.0 mM-pyruvate, the activity of PDC was 80 % of the maximal activity achieved in the presence of these ions and 4.5 mM-pyruvate. The present study suggests that PDC may operate under non-saturating concentrations for substrate *in vivo*.

INTRODUCTION

The activity of the pyruvate dehydrogenase complex (PDC) is regulated by end-product inhibition (Garland & Randle, 1964; Wieland *et al.*, 1969; Tsai *et al.*, 1973) and by interconversion between an active dephosphorylated form and an inactive phosphorylated form by a specific phosphatase/kinase regulatory system (Linn *et al.*, 1969a). The regulatory properties of the phosphatase/kinase system have been studied extensively and shown to be controlled by several agents. The activity of the phosphatase is stimulated by Mg^{2+} and Ca^{2+} (Siess & Wieland, 1972; Denton *et al.*, 1972) and inhibited by NADH (Pettit *et al.*, 1975). Mn^{2+} has an effect similar to Mg^{2+} on the activity of the phosphatase (Siess & Wieland, 1972; Denton *et al.*, 1972). Mg^{2+} , CoA, acetyl-CoA, NADH, ADP and thiamin pyrophosphate (TPP) modulate the activity of pyruvate dehydrogenase kinase, but the extent of these effects depends on the presence of K^+ or NH_4^+ (Pettit *et al.*, 1975; Roche & Reed, 1974; Roche & Cate, 1977; Cooper *et al.*, 1975; Batenburg & Olson, 1976). Furthermore, the activity of the kinase is inhibited by pyruvate, butyrate, propionate and acetate (Linn *et al.*, 1969b; Denton *et al.*, 1975). Conversely, the bivalent metal cations Ca^{2+} , Mn^{2+} , Co^{2+} and Ni^{2+} inhibit the phosphorylation of PDC (Wieland *et al.*, 1973; Schuster & Olson, 1974; Schuster *et al.*, 1975).

In contrast, relatively little is known about the regulation of the activity of PDC by mechanisms other than phosphorylation/dephosphorylation. Previous reports in the literature have shown that K^+ stimulates pyruvate oxidation in rat liver mitochondria (Schaefer & Veneziale, 1973), rat brain mitochondria (Nicklas *et al.*, 1971) and rat brain slices (Kini & Quasted, 1959;

Kovachich & Haugaard, 1977). However, this effect of K^+ on the rate of pyruvate oxidation may be explained by a concomitant decrease in the tissue content of ATP (Kovachich & Haugaard, 1977).

Several previous reports in the literature have shown changes in intracellular ion (K^+ , Na^+ , Cl^- , Ca^{2+}) activities in response to neural and hormonal stimuli (Kamminga *et al.*, 1950; Moore, 1973; O'Doherty & Stark, 1982a,b, 1983; Baumgarten *et al.*, 1984; Wang *et al.*, 1984; Stanton *et al.*, 1986). Disturbances in acid/base balance which occur during various pathophysiological conditions such as diabetes, starvation or acute and chronic renal insufficiency may cause changes in intracellular pH (Jones & Simpson, 1983a,b; Cogan *et al.*, 1981) and redistribution of K^+ and Na^+ between the intra- and extra-cellular spaces (Khuri *et al.*, 1972; DeMello-Aires *et al.*, 1973; Patrick, 1977; Kubota *et al.*, 1983). In addition, renal ischaemia causes changes in intracellular electrolyte composition (Mason *et al.*, 1981).

The present study examines the effects of K^+ , Na^+ , Cl^- , HCO_3^- and HPO_4^{2-} on the activity of PDC purified from pig kidney cortex. The reported effects of anions and cations on PDC activity may be significant in the kidney, where there is a constant, dynamic, passage of ions intracellularly under hormonal regulation. However, on the basis of reports in the literature of physiological changes in intracellular ion activities, it is difficult to estimate the magnitude of such changes in different intracellular spaces, especially in mitochondria. Intracellular changes of ion activities have been reported without consideration of their intracellular compartmentalization, owing to the technical problems in measurement of ionic activities within cells. However, reports in the literature have shown that intracellular ion

Abbreviation used: PDC, pyruvate dehydrogenase complex; TPP, thiamin pyrophosphate.

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heterogeneity is possible (Pietrzyk & Heinz, 1974; Palmer & Civian, 1977; Civian, 1978; Beck *et al.*, 1980).

MATERIALS AND METHODS

Materials

Bovine serum albumin, cysteine hydrochloride, NAD⁺, Hepes, leupeptin, pyruvic acid, Tris, Triton X-100 and Mops were obtained from Sigma. CoA and dithiothreitol were obtained from P-L Biochemicals. TPP was from Calbiochem-Behring. All other chemicals were of the purest grade commercially available.

Methods

Mitochondria were isolated from 5.0 kg of pig kidney cortex by the method of Linn *et al.* (1972) as modified by Roche & Cate (1977). The mitochondria obtained were resuspended in 800 ml of 20 mM-potassium phosphate buffer (pH 6.5)/0.1 mM-EDTA/0.2 mM-TPP supplemented with 20 ml of rabbit serum and frozen rapidly in liquid N₂. The frozen mitochondria were kept at -70 °C for several days without significant loss of PDC activity.

PDC was purified from the isolated mitochondria essentially as described by Roche & Cate (1977), but with the following modifications. The thawed mitochondrial suspension was diluted with 20 mM-potassium phosphate, pH 6.5, before Triton X-100 and leupeptin were added (final concns. 3% and 10 μ M respectively) (Pawelczyk & Angielski, 1984; Machicao & Wieland, 1980). The suspension was centrifuged at 11000 *g* for 30 min. The supernatant obtained was diluted to a protein concentration of 10 mg/ml, and poly(ethylene glycol) was added to precipitate 2-oxoglutarate dehydrogenase complex and PDC (Roche & Cate, 1977). The suspension was centrifuged at 10000 *g* for 20 min. and the precipitate was resuspended in 50 mM-Mops/NaOH (pH 7.0) before clarification by centrifugation at 35000 *g* for 45 min. The supernatant was diluted to a protein concentration of 5 mg/ml, and sufficient polyethylene glycol was added to separate 2-oxoglutarate dehydrogenase activity from

Fig. 1. Effect of Na⁺ (●) and K⁺ (○) on the activity of PDC

Assay conditions for PDC activity were as described in the Materials and methods section; 3.0 mM-pyruvate was used. The results are mean values $\pm$ s.d. from eight determinations, for three different enzyme preparations.

Fig. 2. Effect of K⁺ on the activity of PDC in the presence of Na⁺

Assay conditions for PDC activity were as described in the Materials and methods section. Activity of PDC was measured in the presence of K⁺ alone (○), or K⁺ in the presence of 20 mM-Na⁺ (●), and 3.0 mM-pyruvate. The results are mean values $\pm$ s.d. from six determinations, for three different enzyme preparations.

PDC activity (Roche & Cate, 1977). [During the poly(ethylene glycol) precipitation steps leupeptin was maintained at 10 μ M.] The precipitate containing PDC activity was resuspended in 50 mM-Mops/Tris, pH 7.2, and clarified by centrifugation at 30000 *g* for 45 min. The supernatant obtained was diluted with 50 mM-Mops/Tris, pH 7.2, to a protein concentration of 8 mg/ml, and the enzyme was sedimented by ultracentrifugation as described by Roche & Cate (1977). The precipitate obtained was transferred to 50 mM-Mops/Tris (pH 7.2)/0.05 mM-TPP/0.1 mM-cysteine hydrochloride/0.1 mM-NAD⁺ and stirred gently for 12 h at 4 °C. The solution was diluted to a protein concentration of 5.0 mg/ml with 50 mM-Mops/Tris, pH 6.5, and clarified by centrifugation at 30000 *g* for 45 min. PDC was precipitated by the addition of poly(ethylene glycol) (Roche & Cate, 1977), harvested by centrifugation at 10000 *g* for 10 min at 18 °C, and resuspended in 50 mM-Mops/Tris, pH 7.2, containing 0.01 mM-TPP and 0.1 mM-dithiothreitol. The yellow solution obtained was stirred gently at 4 °C for 6 h and stored in small batches at -70 °C.

The specific activity of purified PDC ranged from 11 to 14 μ mol of NADH/min per mg of protein (measured at 0.04 M ionic strength).

PDC assay

Enzyme activity was assayed by measuring spectrophotometrically the formation of NADH at 340 nm and 30 °C. The reaction mixture (ionic strength 0.13 M) contained Tris/Hepes buffer, pH 7.8, 2.5 mM-NAD⁺, 0.5 mM-CoA, 0.5 mM-TPP, 2.6 mM-cysteine hydrochloride, 1 mM-MgCl₂ and 1.0 μ g of PDC. Pyruvate (K⁺ salt) was added at various concentrations (see the Figure legends). The ions studied were added in concentrations as noted in the Figure legends, and the ionic strength of the mixture was standardized to 0.130 M by varying the concentration of buffer.

A stock solution containing PDC (0.2 mg/ml) in

Fig. 3. Effect of K^+ and Na^+ on the activity of PDC (Hill plot)

Assay conditions for PDC activity were as described in the Materials and methods section. Activity was determined in the presence of: (a) no additions; (b) 80 mM- K^+ (●) and 80 mM- Na^+ (○); (c) 20 mM- K^+ (●) and 20 mM- Na^+ (○); (d) 100 mM- K^+ (●) and 80 mM- K^+ plus 20 mM- Na^+ (○). The results are mean values from three or four determinations.

Tris/Hepes buffer, pH 7.8, 1.0 mM- $MgCl_2$, 2.6 mM-cysteine hydrochloride, 0.5 mM-TPP (ionic strength 0.130 M) was incubated for 45 min at 0 °C before use. The assay reaction was initiated by addition of CoA after a preincubation period of 45 s at 30 °C. The pH of each reaction mixture was monitored carefully (± 0.01 pH unit at 30 °C). HCO_3^- solutions were prepared immediately before use.

Protein was determined by the biuret procedure (Gornall *et al.*, 1949), with bovine serum albumin as standard.

The concentrations of conjugate base and acid for Tris and Hepes buffers were calculated from the Henderson-Hasselbalch equation. The results presented are representative for three enzyme preparations.

RESULTS

Effects of K^+ and Na^+ on the activity of PDC

Incubation of PDC in the presence of K^+ resulted in the stimulation of enzymic activity as compared with control activity determined in the absence of this ion.

The relationship between the activity of PDC and the ion concentration was sigmoidal (Fig. 1). Incubation of the complex with Na^+ also resulted in an increase in activity, but the dependency of this stimulation on ion concentration showed different characteristics at concentrations in the range 0–40 mM. Thus, although K^+ at 20 mM caused an increase in the activity of PDC by 4%, Na^+ at the same concentration activated the enzyme by 11% (Fig. 1). However, both K^+ and Na^+ at 120 mM stimulated the activity of PDC by 37% (Fig. 1).

The effects of K^+ (10–80 mM) and Na^+ (at a constant 20 mM) on the activity of PDC were additive (Fig. 2). Thus, in the presence of 20 mM- K^+ the addition of 20 mM- Na^+ increased the activity of PDC by 17%, but in the presence of 40 mM- K^+ the activity was stimulated by 9% (Fig. 2). However, the addition of Na^+ (20 mM) in the presence of higher concentrations of K^+ (40–100 mM) produced the same effect as the addition of a further 20 mM- K^+ . Thus, for example, in the presence of K^+ and Na^+ at 60 mM and 20 mM respectively, the activity of PDC was increased by 30% relative to the control and was equivalent to the stimulation produced by the

Table 1. Effects of Na⁺ and K⁺ on the kinetic constants of PDC

Assay conditions for PDC activity were as described in the Materials and methods section. Na⁺ and K⁺ were present in the reaction mixture as Hepes salt. The results are mean values $\pm$ S.D. from three determinations.

Additions	Concn. (mM)	$V_{\max}$ (μ mol/min per mg)	$s_{0.5}$ for pyruvate (μ M)	Hill coefficient (h)
None		6.80 $\pm$ 0.15	75 $\pm$ 9	1.3
K ⁺	20	6.98 $\pm$ 0.12	90 $\pm$ 10	1.0, 1.3
K ⁺	80	8.84 $\pm$ 0.19	93 $\pm$ 8	1.1, 1.7
Na ⁺	20	7.55 $\pm$ 0.11	75 $\pm$ 10	1.3
Na ⁺	80	8.77 $\pm$ 0.17	78 $\pm$ 7	1.3
K ⁺	80	9.25 $\pm$ 0.18	93 $\pm$ 11	1.3
Na ⁺	20			
K ⁺	100	9.17 $\pm$ 0.15	87 $\pm$ 10	1.3, 2.0

addition of 80 mM-K⁺ (Fig. 2). These data demonstrate that Na⁺ at 20 mM and in the presence of low concentrations of K⁺ (0–40 mM) has a specific effect on the activity of PDC relative to K⁺, but that this effect is suppressed in higher concentrations of K⁺.

That Na⁺ and K⁺ have different, specific, effects was demonstrated by the differential influence of these ions on the kinetic parameters of PDC activity (Fig. 3). K⁺ caused a change (not statistically significant) in the $s_{0.5}$ for pyruvate and also modified the Hill coefficient, h (Fig. 3). In contrast, Na⁺ increased the $V_{\max}$ of PDC without a concomitant change in the $s_{0.5}$ for pyruvate or in h (Fig. 3, Table 1). In the presence of K⁺ the plot showed a distinct break in linearity (Fig. 3), but the concentration of pyruvate at which this break occurred depended on the concentration of the cation. This result suggests that two classes of binding sites exist on the complex for pyruvate and that the nature of site-site interaction is dependent on the concentration of K⁺.

The change in h owing to K⁺ ions at 80 mM could be prevented by the inclusion of 20 mM-Na⁺ (Table 1). The concentrations of cations used in this experiment were within the physiological range (Greger, 1985; Beck *et al.*, 1980), and therefore it might be speculated that the co-operative effects of Na⁺ and K⁺ on PDC activity result in an increased $V_{\max}$ and $s_{0.5}$ for pyruvate *in vivo*. Furthermore, these results suggest the possibility that the concentration of Na⁺ *in vivo* may maintain a single class of binding sites for pyruvate.

Effect of HPO₄²⁻ on the activity of PDC

The results in Fig. 4 show that HPO₄²⁻ at 40 mM markedly (25%) increased the activity of PDC. When incubated in the presence of HPO₄²⁻ at a concentration within the physiological range (e.g. 10 mM; Veech *et al.*, 1979) the activity of the enzyme complex was increased by 8% (Fig. 4). In the presence of HPO₄²⁻ (0–40 mM) the Hill plot for PDC was linear with a coefficient (h) of 1.1, which represents a small negative change from the value for the enzyme in the control incubation in the presence of buffer only (e.g. 1.3; Table 2). In contrast with the effects of K⁺, it is evident that under these conditions only one class of binding site for pyruvate is present on the complex. As shown in Table 2, HPO₄²⁻ increased the

Fig. 4. Effect of HPO₄²⁻ on the activity of PDC

Assay conditions for PDC activity were as described in the Materials and methods section; 3.0 mM-pyruvate was used. Reaction mixture contained: HPO₄²⁻ as Tris salt (○); HPO₄²⁻ as Na⁺ salt (●); HPO₄²⁻ as K⁺ salt (■). The results are mean values $\pm$ S.D. from four determinations, for two different enzyme preparations.

Table 2. Effect of HPO₄²⁻ on the kinetic constants of PDC

Assay conditions for PDC activity were as described in the Materials and methods section. HPO₄²⁻ was used as Tris salt. The results are mean values $\pm$ S.D. from four determinations.

Addition	Concn. (mM)	$V_{\max}$ (μ mol/min per mg)	$s_{0.5}$ for pyruvate (μ M)	h
None		5.93 $\pm$ 0.12	75 $\pm$ 11	1.3
HPO ₄ ²⁻	10	6.40 $\pm$ 0.11	80 $\pm$ 10	1.1
HPO ₄ ²⁻	40	7.35 $\pm$ 0.09	93 $\pm$ 12	1.1

$V_{\max}$ of PDC and increased (not statistically significant) the $s_{0.5}$ for pyruvate, indicating that these effects are likely to be responsible for the modification of PDC activity by HPO₄²⁻. These data also suggest that HPO₄²⁻ modifies the interaction of the components of PDC, as demonstrated by the apparent decrease in positive co-operativity.

The effect of HPO₄²⁻ on the activity of PDC was inhibited markedly in the presence of K⁺ and Na⁺, although to different extents. The presence of K⁺ (80 mM) almost totally prevented (90%) the stimulatory effect of 40 mM-HPO₄²⁻, whereas Na⁺ (80 mM) inhibited the effect of HPO₄²⁻ by 60% (Fig. 4). This counter-effect of K⁺ and Na⁺ was dependent on the concentration of these two species; at concentrations less than 40 mM these ions had no effect on the stimulation of PDC activity produced by HPO₄²⁻ (Fig. 4).

Effect of HCO₃⁻ and Cl⁻ on the activity of PDC

The activity of PDC was also sensitive to Cl⁻ and HCO₃⁻ (Fig. 5). Both anions decreased the activity of

Fig. 5. Effect of HCO_3^- (●) and Cl^- (○) on the activity of PDC

Assay conditions for PDC activity were as described in the Materials and methods section, with 3.0 mM-pyruvate. The results are mean values $\pm$ S.D. from five determinations, for three different enzyme preparations.

Table 3. Effects of Cl^- and HCO_3^- on the kinetic constants of PDC

Assay conditions for PDC activity were as described in the Materials and methods section. Cl^- was present in the reaction medium as Tris salt. The results are mean values $\pm$ S.D. from three determinations.

Addition	Concn. (mM)	$V_{\max}$ ($\mu\text{mol}/\text{min}$ per mg)	$s_{0.5}$ for pyruvate (μmol)	h
None		7.40 ± 0.12	75 ± 12	1.3
Cl^-	80	5.77 ± 0.19	126 ± 14	1.3
K^+	80	9.55 ± 0.17	93 ± 10	1.1, 1.7
KHCO_3	80	8.40 ± 0.20	100 ± 11	1.2

PDC in a concentration-dependent manner, but the inhibitory effect of Cl^- was significantly stronger (Fig. 5): in the presence of 80 mM- Cl^- the activity of PDC decreased by 22%, whereas HCO_3^- at the same concentration inhibited the enzyme by only 12%. The inhibition of the activity of PDC caused by Cl^- and HCO_3^- was additive (Table 4). Further analysis showed that the addition of 80 mM- Cl^- markedly increased the $s_{0.5}$ for pyruvate from 75 μM , in the absence of Cl^- , to 125 μM (Table 3). This result suggests that the inhibitory effect of Cl^- is the consequence of a decreased affinity of the complex for pyruvate. Furthermore, the maximum enzyme activity was also decreased after addition of this anion (Table 3).

The addition of HCO_3^- (80 mM) in the presence of K^+ (80 mM) modulated h and also caused a marginal increase in the $s_{0.5}$ for pyruvate (Table 3). This result suggests that a co-operative effect exists between K^+ and HCO_3^- , resulting in an increase in the $V_{\max}$ of PDC and

Table 4. Effects of K^+ , Na^+ , Cl^- , HCO_3^- and HPO_4^{2-} on the activity of PDC

Assay conditions for PDC activity were as described in the Materials and methods section, with 3.0 mM-pyruvate. The results are mean values $\pm$ S.D. from four determinations, for three different enzyme preparations.

Additions	Concn. (mM)	PDC activity (% of control)
None	—	100
Cl^-	20	93 ± 3
HCO_3^-	20	96 ± 2
HPO_4^{2-}	10	111 ± 2
Cl^-	20	89 ± 4
HCO_3^-	20	
K^+	80	136 ± 2
Na^+	20	
Cl^-	20	125 ± 2
HCO_3^-	20	
HPO_4^{2-}	10	$*131 \pm 2$
K^+	80	
Na^+	20	

* In this case 5.0 mM-pyruvate was used.

Fig. 6. Effect of K^+ , Na^+ , HPO_4^{2-} , Cl^- and HCO_3^- on the activity of PDC

Assay conditions were as described in the Materials and methods section. Activity was determined in the presence of: no additions (○); 10 mM- HPO_4^{2-} , 80 mM- K^+ , 20 mM- Na^+ , 20 mM- Cl^- , 20 mM- HCO_3^- (●). The results are mean values from three determinations. Insert: the Hill plot.

an increase in the $s_{0.5}$ for pyruvate, as well as a small decrease in h .

Co-operation of K^+ , Na^+ , HPO_4^{2-} , HCO_3^- and Cl^- in the regulation of PDC

A summary of the effects of K^+ , Na^+ , HPO_4^{2-} , HCO_3^- and Cl^- ions at concentrations that are considered close to physiological values (Greger, 1985; Maffly, 1981) on the activity of PDC is presented in Table 4. In the presence of these ions at 80 mM, 20 mM, 10 mM, 20 mM and 20 mM respectively, the activity of PDC was increased by 30%. The $s_{0.5}$ for pyruvate was determined to be

158 μM (compared with the control value of 75 μM ; see Fig. 6) and h under these conditions was 1.1, compared with a control value of 1.3 (Fig. 6). The data obtained indicate that the co-operative effects of K^+ , Na^+ , HPO_4^{2-} , HCO_3^- and Cl^- result in a marked increase in the V_{max} (Table 4) and $s_{0.5}$ for pyruvate (Fig. 6) and also a negative change in h .

DISCUSSION

The studies presented in this paper indicate that the activity of PDC purified from pig kidney cortex is affected by several uni- and bi-valent anions. At constant ionic strength (0.13 M) and pH 7.8, K^+ , Na^+ and HPO_4^{2-} stimulate the activity of PDC, whereas Cl^- and HCO_3^- ions inhibit.

The stimulatory effects of Na^+ (0–80 mM) and K^+ (0–80 mM) were similar. However, Table 1 shows that the mechanism by which Na^+ influences the activity of PDC must result in the increase in the maximum activity of the enzyme (V_{max}) only, whereas the mechanism involved in the effect of K^+ must result in an increase in h as well as in the V_{max} on the activity of PDC. The differential effects of these ions may indicate that two separate and specific classes of binding sites or regions exist for Na^+ and K^+ . Furthermore, the larger stimulatory effect of Na^+ on the activity of PDC compared with K^+ when added in low concentrations (0–40 mM) could be the result of a greater affinity of Na^+ for its specific binding site.

Although the specific mechanisms of action of Na^+ or K^+ are unclear, it is proposed that the binding of Na^+ to specific sites causes a change in the conformation of the PDC, which, in turn, increases the number of binding sites for pyruvate (increase in V_{max}) without affecting the affinity for pyruvate ($s_{0.5}$) or site-site interactions (h). Conversely, K^+ could produce similar conformational changes which result in an increase in the number of binding sites for substrate (V_{max}) with the simultaneous increase in h . This scenario would result in the creation of two classes of binding sites or regions with different site-site interactions (h_1 , h_2) and would account for the effect of K^+ on h .

If our hypothesis is correct, then the difference in the ability of K^+ and Na^+ to inhibit the effect of HPO_4^{2-} on the activity of PDC (Fig. 4) could be explained by different conformational changes produced after the binding of these ions. However, when PDC is incubated with K^+ and Na^+ in concentrations within the physiological range (Greger, 1985; Maffly, 1981), no effect of HPO_4^{2-} (10 mM) is evident (Table 4).

The resultant effects of K^+ , Na^+ , HPO_4^{2-} , Cl^- and HCO_3^- ions on the activity of PDC (in the concentrations given in Table 4) were observed as increases in the V_{max} (Table 4), $s_{0.5}$ for pyruvate and h (Fig. 6). Under these conditions and at 1.0 mM-pyruvate, the activity of PDC was 80% of the maximal activity as determined in the presence of these ions at 4.5 mM-pyruvate (Fig. 6). If it can be assumed that the concentration of pyruvate in intact mitochondria *in situ* is close to 1.0 mM (Siess *et al.*, 1977) and that the anion and cation effects defined in the present study were performed at ion concentrations similar to actual physiological values, it is likely that PDC is not saturated with substrate under normal conditions *in situ*. Under such conditions the kidney enzyme complex may be regulated not only by a

phosphorylation/dephosphorylation system and end-product inhibition, but also via relatively small changes in the concentration of substrate.

This work was supported by grants from N.I.H. (HL-24654) and the Robert A. Welch Foundation (AQ-728).

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Received 19 October 1987/15 January 1988; accepted 12 February 1988